THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

JOINT MEETING

A JOINT Meeting of the Society with the Fine Chemicals Group of the Society of Chemical Industry was held at 7 p.m. on Friday, March 22nd, 1957, in the Chemistry Lecture Theatre, King's College, London, W.C.2. The Chair was taken by the President of the Society for Analytical Chemistry, Dr. J. H. Hamence, M.Sc., F.R.I.C.

The following paper was presented and discussed: "Organic Reagents in Inorganic Analysis: Some Recent Developments," by H. M. N. H. Irving, M.A., D.Phil., F.R.I.C., L.R.A.M.

ORDINARY MEETING

AN Ordinary Meeting of the Society, organised by the Physical Methods Group, was held at 7 p.m. on Wednesday, April 3rd, 1957, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. J. H. Hamence, M.Sc., F.R.I.C.

The following papers were presented and discussed: "The Spectrometry of Fluorescence," by E. J. Bowen, M.A., D.Sc., F.R.S.; "Some Experiments with Spectrofluorimeters and Filter Fluorimeters," by C. A. Parker, B.Sc., Ph.D., F.R.I.C.; "Spectrofluorimetry," by Professor R. T. Williams, Ph.D., D.Sc.; "A Direct-reading Fluorimeter," by L. Brealey, B.Sc., and R. E. Ross, A.M.Brit.I.R.E.

NEW MEMBERS

ORDINARY MEMBERS

Shelagh Maureen Burns, B.Pharm. (Nott.), M.P.S.; Ethel Neil, B.Sc. (Dunelm.); Patricia Deloraine Parr-Richard, B.Sc. (Lond.), A.R.C.S.

JUNIOR MEMBER

Muriel Cessford Gray, B.Sc. (Edin.).

DEATH

WE record with regret the death of

Douglas Arnold Yoxall.

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held at 7.15 p.m. on Friday, February 22nd, 1957, in the Central Hotel, Glasgow. The Chair was taken by the Vice-Chairman of the Section, Mr. A. N. Harrow, A.H.-W.C., F.R.I.C.

The following paper was presented and discussed: "Some Recent Developments in Analytical Chemistry," by R. Belcher, Ph.D., D.Sc., F.Inst.F., F.R.I.C.

AN Ordinary Meeting of the Section was held at 7 p.m. on Wednesday, March 13th, 1957, in the George Hotel, George Street, Edinburgh. The Chair was taken by the Chairman of the Section, Dr. Magnus Pyke, F.R.I.C., F.R.S.E.

The following papers were presented and discussed: "Some Aspects of the Estimation of Uronic Acid in Carbohydrate Materials," by D. M. W. Anderson, B.Sc., Ph.D., A.R.I.C.; "The Routine Semi-micro Determination of Molecular Weights," by J. Brooks, M.A., A.R.I.C., and A. F. Williams, B.Sc., F.R.I.C.

WESTERN SECTION

AN Ordinary Meeting of the Section was held at 6.30 p.m. on Friday, February 22nd, 1957, at the College of Technology, Ashley Down, Bristol. The Chair was taken by the Chairman of the Section, Mr. P. J. C. Haywood, B.Sc., F.R.I.C.

A lecture on "The Oxygen Demand of Trade Effluents with Respect to River Pollution" was given by C. J. Regan, B.Sc., F.R.I.C.

A JOINT Meeting of the Section with the South Wales Section of the Royal Institute of Chemistry was held at 6.30 p.m. on Friday, March 15th, 1957, in the Chemistry Lecture Theatre, University College, Swansea. The Chair was taken by the Chairman of the Section, Mr. P. J. C. Haywood, B.Sc., F.R.I.C.

A lecture on "Some Recent Developments in Metallurgical Analysis" was given by G. W. C. Milner, M.Sc., A.Inst.P., F.R.I.C.

MIDLANDS SECTION AND PHYSICAL METHODS GROUP

A JOINT Meeting of the Midlands Section and Physical Methods Group was held at 7 p.m. on Tuesday, February 12th, 1957, in the English Theatre, The University, Edmund Street, Birmingham, 3. The Chair was taken by the Chairman of the Physical Methods Group, Dr. J. E. Page, F.R.I.C.

The Meeting took the form of a discussion on "High-frequency Titrations" and the subject was introduced as follows: "Instrumentation," by J. Allen, A.R.I.C.; "Applications," by E. S. Lane, B.Sc., Ph.D., F.R.I.C.

MIDLANDS SECTION

A JOINT Meeting of the Section with the Birmingham and Midlands Branch of the Royal Institute of Chemistry was held at 7 p.m. on Tuesday, March 5th, 1957, in the Main Chemistry Theatre, The University, Edgbaston, Birmingham, 15. The Chair was taken by the Chairman of the Midlands Section, Dr. R. Belcher, F.Inst.F., F.R.I.C.

A lecture on "Thermo-gravimetric Analysis" was given by Professor C. Duval (Paris).

MICROCHEMISTRY GROUP

THE Thirteenth Annual General Meeting of the Group was held at 6.45 p.m. on Friday, January 25th, 1957, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chairman of the Group, Dr. G. F. Hodsman, A.Inst.P., presided. The following Officers and Committee Members were elected for the forthcoming year:—*Chairman*—Mr. D. F. Phillips. *Vice-Chairman*—Mr. F. Holmes. *Hon. Secretary*—Mr. D. W. Wilson, Department of Chemistry, Sir John Cass College, Jewry Street, Aldgate, London, E.C.3. *Hon. Treasurer*—Mr. G. Ingram. *Members of Committee*—Mrs. D. Butterworth and Messrs. R. A. Chalmers, R. Goulden, G. F. Hodsman, W. I. Stephen and C. L. Wilson. In order to adjust the number of Committee Members retiring each year, two Members, Messrs. Hodsman and Stephen, were elected to serve for one year only. Dr. L. H. N. Cooper and Mr. H. Childs were re-appointed as Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Society, organised by the Group.

THE ninth London Discussion Meeting of the Microchemistry Group was held at 6.30 p.m. on Wednesday, February 27th, 1957, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by Dr. G. F. Hodsman, A.Inst.P.

A discussion on "The Micro-determination of Halogens" was opened by F. Oliver and R. Goulden, A.R.I.C.

BIOLOGICAL METHODS GROUP

AN Ordinary Meeting of the Group was held at 6.30 p.m. on Wednesday, March 6th, 1957, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Chairman of the Group, Dr. S. K. Kon, F.R.I.C.

The Meeting took the form of a discussion on "The Experimental Assessment of Tranquillisers," which was opened by A. Spinks, B.A., B.Sc., Ph.D., D.I.C.

The Potentiometric Titration of Weak Acids and Bases in Dilute Aqueous Solution*

By J. C. GAGE

The limitations in the application of the Henderson equation to the titration of weak acids and bases is discussed. It is shown that the graphical method of finding the concentration, c, and the (Brønsted) dissociation constant, K, may be given a slightly enlarged range of usefulness by means of the exact form of the Henderson equation. Of more general application is the transformation of the titration curve to a linear form; this permits the determination of c and K when one or both end-point inflexions are obscured, provided that c is not less than K or K_w/K. Examples are given from the titration of chlorophenols at a concentration of 10^{-4} M, singly and in admixture, and of certain alkaloids.

THE usual procedure for the quantitative and qualitative determination of weak acids and bases by means of potentiometric titration is based on the Henderson equation, which is presented in equation (1) in the form for the titration of a weak acid with a strong alkali; c is the concentration of the acid, m the concentration of alkali, and

$$\mathrm{pH} = \mathrm{p}K + \log_{10}\left[\frac{m}{c-m}\right] \quad .. \quad .. \quad .. \quad .. \quad (1)$$

pK is the negative logarithm of the (Brønsted) dissociation constant, K. This equation is an approximation and involves the assumption that the hydrogen-ion concentration, h, and the hydroxyl-ion concentration, K_w/h, are negligible in comparison with m; in the exact equation m must be replaced by $(m + h - K_w/h)$. According to equation (1), the titration curve should be asymptotic to $m = 0$, $m = c$, but at high and low pH values the assumption that h and K_w/h are negligible is no longer valid and the ends of the curve are flattened, the asymptotes being replaced by points of inflexion. In the application of equation (1) to the potentiometric titration of weak acids, it is assumed that these points of inflexion correspond to the end-points of the titration, and the pH of the point half-way between them gives the pK value of the acid; a precise mathematical study of the potentiometric-titration curve, which is at present being prepared for publication, reveals that none of these assumptions is entirely justified and that the systematic error in making them increases with increasing dilution.

Equation (1) does not suggest that the scope and precision of potentiometric titration should be in any way affected by the value of pK, or by the dilution provided that the precision in determining the ratio m/c is not thereby affected. The experimental difficulty in the determination of pK and c of weak acids and bases at low concentration is due to the deviation of the titration curve from that expected from equation (1). The flattening of the curve becomes more pronounced as the hydrogen and hydroxyl-ion concentrations approach m; as the dilution increases or as the pK value departs from 7, one or both end-point inflexions become progressively less well defined, resulting in a progressive loss of precision until a point is reached at which the inflexion is no longer visible. This is demonstrated in the titration curves for *p*-chlorophenol and 2:4-dichlorophenol shown in Fig. 1, curves A and B, at a concentration in the region of 10^{-4} M; the weak inflexion on curve B appears as a shallow peak on the differential curve of $d(\mathrm{pH})/dm$ against m, while no inflexion is apparent in curve A, nor any peak in the corresponding differential curve. If two acids are present in the solution titrated, it may be impossible to measure graphically their separate concentrations, as is shown in Fig. 6 (p. 226) for a mixture of 2:4:5-trichlorophenol and 2:3:4:6-tetrachlorophenol, each approximately 10^{-4} M, although the pK values of these two phenols differ by 0·76 unit.

The theory of potentiometric titration of acids and bases, singly and in admixture, has been studied by Auerbach and Smolczyk[1] and has been further extensively developed

* Presented at the XVth International Congress on Pure and Applied Chemistry (Analytical Chemistry), Lisbon, September 8th to 16th, 1956.

by Ricci,[2] who includes a mathematical treatment of the feasibility of a titration. These authors do not, however, consider what information can be extracted from a titration curve when the graphical method is impossible or can be shown to lead to unacceptably large errors.

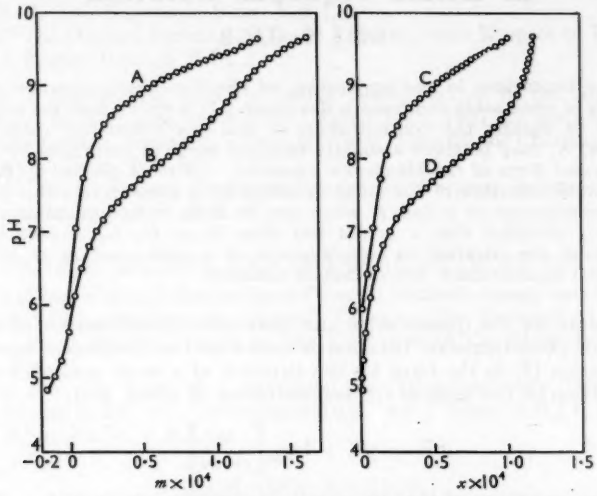

Fig. 1. Potentiometric-titration curves: curve A, pH - m for p-chlorophenol; curve B, pH - m for 2:4-dichlorophenol; curve C, pH - x for p-chlorophenol; curve D, pH - x for 2:4-dichlorophenol

The precision of the potentiometric titration of weak acids and bases, particularly of those with a low aqueous solubility, may be increased by substituting a suitable polar organic solvent, such as acetic acid or pyridine, for water. Although this procedure may permit an accurate quantitative determination, it is of limited value when the substance to be investigated is only available in aqueous solution. Moreover, it is not possible to calculate from a titration in a non-aqueous solvent the dissociation constant in aqueous solution. The importance of a knowledge of the dissociation of acids and bases under physiological conditions, in the study of the relation between chemical structure and pharmacological action, has been emphasised by many investigators, particularly by Albert.[3] During a comparative study in these laboratories of the biological action of a series of chlorinated phenols, it became evident that a knowledge of their dissociation constants would be desirable. Although figures for all of the substituted phenols under investigation had been published, the methods used varied widely, and an attempt was made to develop a standard procedure that could be applied to the whole series. Potentiometric titration is an attractive method, as it is simple to perform and should be applicable to substances of unknown purity, but the wide range of acidities of the series of chlorinated phenols, and the low solubility of some of its members, necessitated a re-investigation of the mathematical treatment of the results of potentiometric titration.

Theoretical treatment

Titration of single acids—

The general equation for the titration of a weak acid by a strong alkali is given by (2). This may more conveniently be expressed in the form (3), where $m + h - K_w/h = x$. Equation (3) may be written as (4), which has the same form as (1), but it is an exact and

$$\frac{h}{K} = \frac{c-m-h+K_w/h}{m+h-K_w/h} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$\frac{h}{K} = \frac{c-x}{x} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

$$\mathrm{pH} = \mathrm{p}K + \log_{10}\left[\frac{x}{c-x}\right] \quad .. \quad .. \quad .. \quad .. \quad (4)$$

not an approximate equation, and, if the pH values from a potentiometric titration are plotted against x instead of against m, the curve obtained is asymptotic to $x = 0$, $x = c$. In the titration of a single acid the value of x cannot exceed c, and can only be made to approach this value when m is in excess of c and it is, therefore, necessary to add more than one equivalent of alkali to the acid in order to obtain the end-point on the pH - x curve. Similarly when $m = 0$, $x = h - K_w/h$, and in order to obtain the lower end of the curve it is necessary to titrate the weak acid with a strong acid. This is shown in Fig. 1; in curve B positive values of m correspond to the concentration of sodium hydroxide and negative values to the concentration of hydrochloric acid. It is evident from curve D, which has been derived from curve B, that the end-point inflexion on the pH - x curve is much more clearly defined than on the pH - m curve.

TITRATION OF MIXTURES OF ACIDS—

If more than one acid is present, the inflexions at the end-points of the pH - x curve may not be clearly defined, interfering with the accurate determination of c and pK. The general equation for the titration of a mixture of weak acids by a strong alkali is given in equation (5), in which c_1, c_2, c_3 .... and K_1, K_2, K_3, ... are the concentrations and dissociation constants of the component acids. Equation (5) does not permit a formal solution unless certain simplifications are possible.

$$x = \frac{c_1K_1}{K_1 + h} + \frac{c_2K_2}{K_2 + h} + \frac{c_3K_3}{K_3 + h} + \ldots\ldots \quad .. \quad .. \quad .. \quad (5)$$

If $K_1 \simeq h$, and K_2, K_3 ....$\ll h$, all the terms on the right-hand side of (5), except for the first, vanish, leaving an equation that may be converted to (3). As the presence of the other acids may prevent the accurate graphical evaluation of c_1 and pK_1 from the titration curve, equation (3) may be converted to the linear form (6), from which it is evident that the values of $1/K_1$ and c_1 may be found by plotting xh against x. Fig. 2 shows such a plot of xh against

$$\frac{xh}{K_1} + x = c_1 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

x derived from the values of pH and x for 2:4:5-trichlorophenol presented in Fig. 3, curve A.

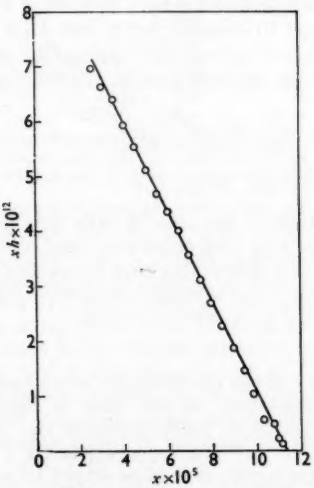

Fig. 2. xh - x curve for 2:4:5-trichlorophenol

If $h \simeq K_1$, and K_2, K_3 ...$\gg h$, equation (5) reduces to (7), which may be written in the form (8). If now xh is plotted against x, a straight line is not obtained and this has been

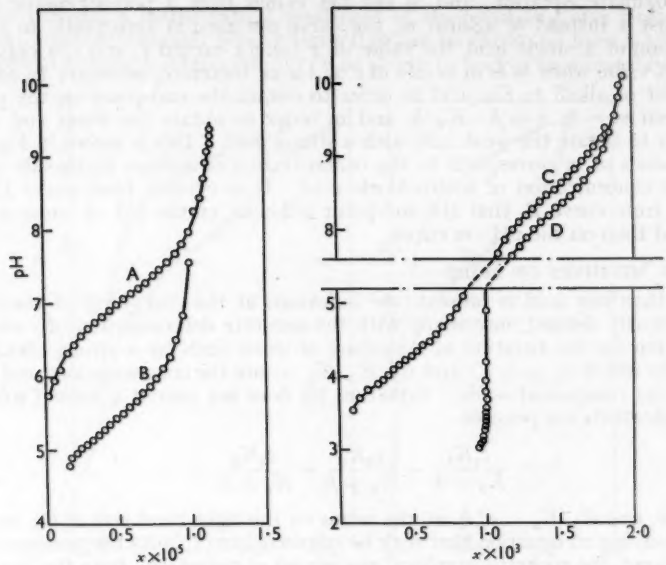

Fig. 3. pH-x curves: curve A, 2:4:5-trichlorophenol; curve B, 2:3:4:6-tetrachlorophenol; curve C, quinine; curve D, brucine

$$x = \frac{c_1 K_1}{K_1 + h} + c_2 + c_3 \ldots\ldots \quad (7)$$

$$x - p = \frac{c_1 K_1}{K_1 + h} \quad (8)$$

observed with most of the chlorophenols studied, indicating their lack of purity. Equation (8) cannot be directly converted to a linear form, but after differentiation (9) can be rearranged to give (10), in which $z = \sqrt{-dh/dx}$. It can be shown that the curve obtained by plotting z against h from the potentiometric titration of a mixture of two acids is

$$-\frac{dx}{dh} = \frac{c_1 K_1}{(K_1 + h)^2} \quad (9)$$

$$z\sqrt{c_1 K_1} = K_1 + h \quad (10)$$

compounded from the intersection of the lines of each acid separately and may permit the separate determinations of the two concentrations and dissociation constants, provided that each component is present as a sufficiently large proportion of the total, and the constants are sufficiently widely separated. The several dissociation constants of a polybasic acid or polyacid base may be determined in a similar manner.

TITRATION OF BASES—

The mathematical treatment of the titration of a weak base by a strong acid is similar to that outlined above. The dissociation of the base is regarded as that of the cation, $BH^+ \rightleftharpoons B + H^+$, and no distinction is made between the constants of acids and bases. In the following equations $x' = [A^-] + K_w/h - h$, where $[A^-]$ is the concentration of strong acid used in the titration; alternatively, if it is preferred to add an equivalent or excess of strong acid to the weak base, followed by back-titration with strong alkali, x' may be replaced by $[A^-] - x$, where x has the previously assigned definition.

For the titration of a single weak base, equation (4) is transformed to (11), while for a mixture of bases, when $K_1 \doteq h$ and $K_2, K_3, \ldots\ldots \gg h$, equation (6) becomes (12). The differential equation (10) is unchanged and applies to both acids and bases.

$$\mathrm{pH} = \mathrm{p}K + \log_{10}\frac{c-x'}{x'} \quad \ldots\ \ldots\ \ldots\ \ldots \quad (11)$$

$$\frac{x'K_1}{h} + x' = c_1 \quad \ldots\ \ldots\ \ldots\ \ldots\ \ldots \quad (12)$$

Experimental

The potentiometric titrations were performed in the apparatus shown in Fig. 4. Into the titration cell, A, dips a glass electrode, B, and the saturated-calomel half-cell, C, is connected through the hair capillary, D. The junction of the saturated potassium chloride solution at D was freshly made before each titration by allowing the solution to enter A from the reservoir E; this was achieved by rotating E until the ridge F coincided with the hole G in the cone of the standard taper joint, as shown in the diagram. During the titration, E was rotated to isolate the reservoir.

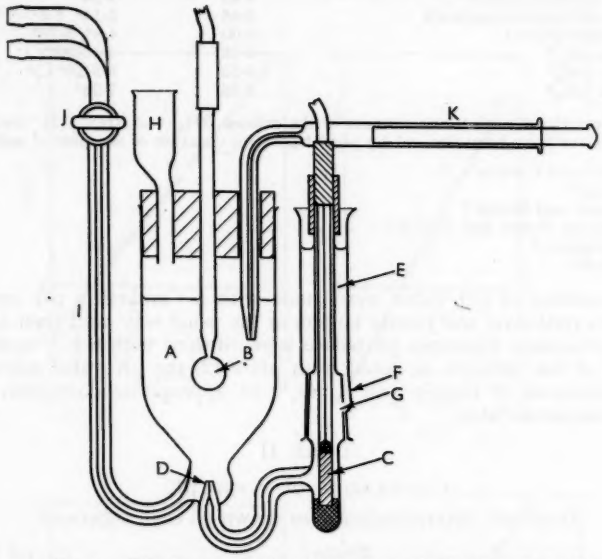

Fig. 4. Titration assembly: A, titration cell; B, glass electrode; C, saturated-calomel half-cell; D, hair capillary; E, reservoir; F, ridge; G, hole; H, funnel; I, tube; J, three-way tap; K, glass syringe

Liquid or washing water may be introduced into the cell through the funnel, H, and the cell is emptied by applying suction to tube I. This tube carries a three-way tap, J, the second arm of which is used to introduce a stream of pure nitrogen into the cell, A, to mix the contents during titration and to protect against the ingress of atmospheric carbon dioxide. The titration cell was immersed in a water bath at 25° C, and the nitrogen, before entering the cell, was bubbled through water maintained at the same temperature.

The titrant was 0·1 *N* sodium hydroxide for the chlorophenols and *N* sodium hydroxide for the alkaloids; it was contained in the glass syringe, *K*, to which is fused a thick-walled glass capillary tip. The solution was expelled from the syringe by means of an Agla micrometer head, not shown in the diagram. This arrangement permits accurate additions of 0·002 ml; 0·1 *N* sodium hydroxide added in this manner to 40 ml of solution is equivalent to a concentration increment of 5×10^{-6} *M*.

The substances titrated are listed in Table I. All were commercially available materials, and with the exception of pentachlorophenol, which was recrystallised before use, were not subjected to purification. A 4×10^{-2} *M* ethanolic solution was prepared from each of the chlorophenols, and 0·1-ml portions of these solutions were added to 40 ml of water to give approximately 10^{-4} *M* concentrations for titration. For the alkaloids brucine and quinine

4×10^{-3} M aqueous solutions were prepared with two equivalents of mineral acid, and 10-ml portions were diluted to 40 ml with water to give 10^{-3} M concentrations.

TABLE I

CALCULATED pK VALUES COMPARED WITH PREVIOUSLY PUBLISHED FIGURES (ALL MEASUREMENTS AT 25° C UNLESS OTHERWISE STATED)

Substance	Calculated pK†	Value from literature
o-Chlorophenol	8·65	8·49[a], 7·44[b]
m-Chlorophenol	9·12	8·85[a], 9·07[c]
p-Chlorophenol	9·37	9·18[a]
2:4-Dichlorophenol	7·85	7·74[a], 7·51[b], 7·8[d]
2:6-Dichlorophenol	6·91	6·8[a]
2:3:6-Trichlorophenol	5·98	6·13[b]
2:4:5-Trichlorophenol	7·07	7·0[d]
2:4:6-Trichlorophenol	6·22	6·41[b], 6·1[d]
3:4:5-Trichlorophenol	7·83	8·35[b]
2:3:4:6-Tetrachlorophenol	5·46	5·14[b], 5·3[d]
Pentachlorophenol	5·00	4·8[d], 5·26[b]
Quinine pK_1*	4·13	4·15(20° C)[e]
pK_2*	8·52	8·3(20° C)[e]
Brucine pK_2*	8·28	7·96[f]

* K_1 and K_2 are the dissociation constants of the cations $BH_2^{++} \rightleftharpoons BH^+ + H^+$ and $BH^+ \rightleftharpoons B + H^+$.
† These pK values have been derived by potentiometric titration of samples of unknown purity.

a. Murray and Gordon.[4]
b. Tiessens.[5]
c. Hodgson and Smith.[6]
d. Blackman, Parke and Garton.[7]
e. Christophers.[8]
f. Kolthoff.[9]

The measurements of pH value were made with a Cambridge pH meter. This was standardised with phthalate and borate buffers in the usual way, and then as a check 25 ml of 8×10^{-4} M potassium hydrogen phthalate were titrated with 0·1 N sodium hydroxide; if the mid-point of the titration deviated from pH 5·42, the pK value calculated from the accurate determinations of Hamer and Acree,[10] an appropriate correction was applied to the subsequent measurements.

TABLE II

CALCULATION OF c AND pK

Duplicate determinations are shown on each substance

Substance	10^4c by— inspection from pH - m curves	10^4c by— inspection from pH - x curves	10^4c by— means of equation (10)	pK by— inspection from pH - m curves	pK by— inspection from pH - x curves	pK by— means of equation (10)
2:4-Dichlorophenol	1·20	1·19	1·13	7·85	7·86	7·93
	1·10	1·12	1·03	7·85	7·83	7·94
2:6-Dichlorophenol	1·08	1·08	1·16	6·93	6·91	6·84
	1·12	1·15	1·18	6·87	6·91	6·87
2:4:5-Trichlorophenol	1·10	1·12	1·11	7·07	7·10	7·05
	1·10	1·14	1·12	7·02	7·07	7·07
2:4:6-Trichlorophenol	1·10	1·12	0·86	6·24	6·23	6·24
	1·12	1·14	0·88	6·19	6·20	6·21
3:4:5-Trichlorophenol	1·00	1·05	1·01	7·92	7·93	7·82
	1·00	1·02	1·04	7·94	7·94	7·84

RESULTS

Table II shows values of pK and c derived by inspection of the pH - m curves, by inspection of the pH - x curves and by means of equation (10). Only the values for two dichlorophenols and three trichlorophenols are included in this Table; with the remainder of the chlorophenols studied, one end-point was unobtainable from the pH - m curve, and was subject to considerable uncertainty on the pH - x curve.

From the pH - x curve for 2:4:5-trichlorophenol, shown in Fig. 3, curve A, has been derived the plot of xh against x shown in Fig. 2. From the parameters of this straight line, c and pK have been calculated to be $1{\cdot}13 \times 10^{-4}$ M and 7·09 by equation (6); these values agree well with those in Table II.

In the derivation of c and K by means of equation (10), the value of z^2 may be obtained graphically from a plot of h against x, but more simply and with no loss of accuracy by deriving $\triangle h$ and $\triangle x$ from the differences between alternate values of these variables. This is illustrated in Table III, which shows the stages in the calculation of z from some of the measured values of pH and m for 2:4:5-trichlorophenol. Four examples of h - z curves are given in Fig. 5; curves A and B are derived from the pH - x curves A and B in Fig. 3 for 2:4:5-trichlorophenol and 2:3:4:6-tetrachlorophenol, and curves C and D represent the two titration steps of the pH - x curve for quinine in Fig. 3, curve C, from which the two dissociation constants may be calculated. The values of pK, calculated by means of equation (10), for all of the substances examined are listed in Table I, together with pK values taken from the literature.

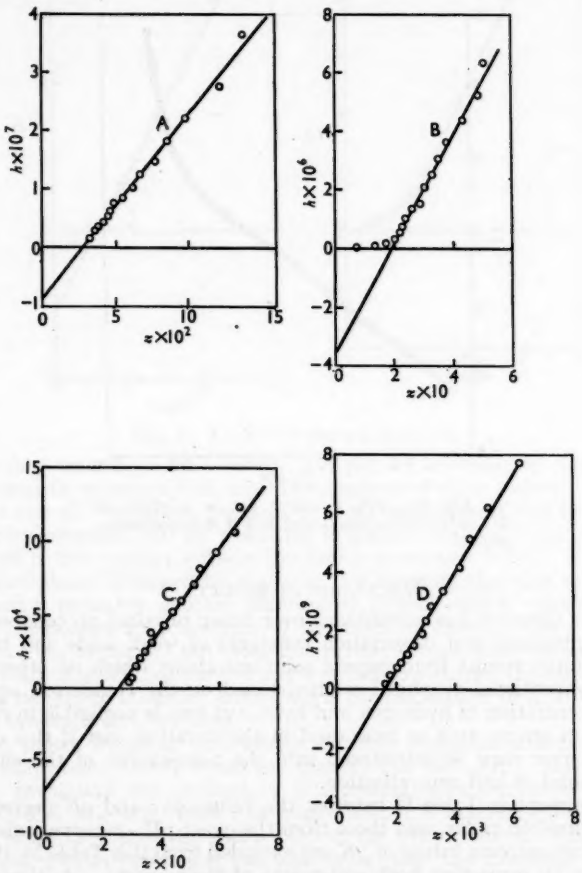

Fig. 5. h-z curves: curve A, 2:4:5-trichlorophenol; curve B, 2:3:4:6-tetrachlorophenol; curves C and D, quinine

Fig. 6 shows the pH - x curve for a mixture of 2:4:5-trichlorophenol and 2:3:4:6-tetrachlorophenol, each 10^{-4} M. Fig. 7, curve B, is the plot of h against z for this mixture; the lower end of this curve has been plotted with extended co-ordinates in Fig. 7, curve A.

TABLE III

CALCULATION OF $z = \sqrt{-\Delta h/\Delta x}$ FROM pH AND m FOR 2:4:5-TRICHLOROPHENOL

pH	$10^5 m$	$10^5 h$	$10^5 x$	$-10^5 \Delta h$	$10^4 \Delta x$	$z = 10^2\sqrt{-\Delta h/\Delta x}$
6·74	3·5	18·2	3·52			
6·83	4·0	14·8	4·01	5·9	9·9	7·7
6·91	4·5	12·3	4·51	4·6	10·0	6·8
6·99	5·0	10·2	5·01	3·8	9·9	6·2
7·14	6·0	7·25	6·00	2·35	9·9	4·85
7·21	6·5	6·15	6·49	2·1	9·8	4·65
7·29	7·0	5·15	6·98	2·0	9·9	4·5
7·38	7·5	4·15	7·48			

The close resemblance between curves A and B of Figs. 5 and 7 will be apparent; the pK values calculated from Fig. 7, curves A and B, differ by less than 2 per cent. from those derived from Fig. 5, curves A and B, while the concentrations are less accurate, being within 15 per cent. of the expected value.

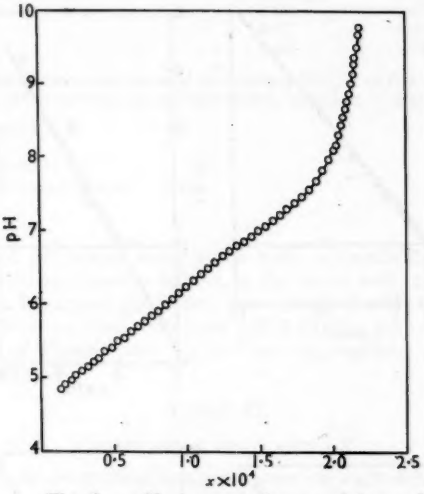

Fig. 6. pH - x curve for a mixture of 2:4:5-trichlorophenol and 2:3:4:6-tetrachlorophenol

DISCUSSION OF RESULTS

Potentiometric titration has advantages over other physical procedures for the determination of concentrations and dissociation constants of weak acids and bases, in that it may yield the required results from impure solutions about which no other information is available. The conventional graphical method based on the Henderson equation (1) may be used if the concentration of hydrogen and hydroxyl ions is negligible in comparison with the concentration of strong acid or base used in the titration, but if this condition is not fulfilled a serious error may be introduced into the assessments of the end-points of the titration and the point of half neutralisation.

The close agreement in Table II between the values of c and pK derived by the usual method from the titration curve, and those from the exact pH - x curve, exists only because substances with more extreme values of pK are excluded from this Table by the impossibility of directly defining by inspection both end-points of their curves. A comparison between the pH - m and pH - x curves for p-chlorophenol in Fig. 1, curves A and C, indicates that in the region of half neutralisation the former curve has a pH value about 0·2 units lower than the latter.

Although the error implicit in the Henderson equation should be avoided by means of the exact form (4), it has been found in practice that this transformation of the titration

curve does not greatly extend the graphical method of solution; when h or K_w/h approaches or exceeds m in value, the evaluation of x is subject to considerable errors arising from the magnification of minor errors in the standardisation of the pH meter, or of minor deviations from linearity of response. This uncertainty in the location of the end-point may be avoided by converting the general equation to a linear form, and this has been effected by equations (6) and (10); the latter equation is of more general application and several examples of its use are presented here. As (10) is a differential equation, the experimental plot cannot be expected to yield the required parameters with as much accuracy as would the Henderson equation when this is admissable. Nevertheless, the graphs shown in Fig. 5 demonstrate that the scatter of the points is not so large as to involve an unacceptably large error in defining the slope and position of the straight line and, therefore, in the calculated values of c and K.

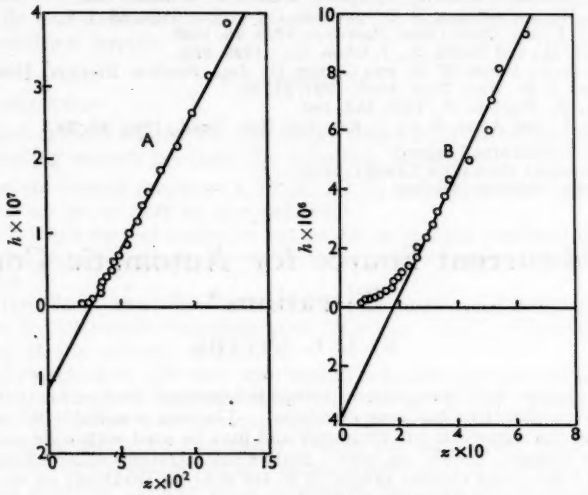

Fig. 7. h - z curves derived from Fig. 6

The reasonably close agreement shown in Table II between the duplicate values of c and pK determined by equation (10), and a comparison of these values with those obtained by inspection of the pH - x curves, suggests that no serious error will be involved in those values obtained by equation (10) for which the inspection method is not available, provided that this method is not applied outside the limits prescribed below. The low value of c for 2:4:6-trichlorophenol obtained by equation (10) suggests that this substance contains a titratable impurity, probably another chlorinated phenol, which cannot be distinguished from the main component by inspection of the titration curve. The h - z curve gives an indication of such an impurity, but does not permit its pK value to be ascertained. In general, the derivation of K from the h - z curve, which is obtained directly from the intercept on the h axis, is subject to a smaller error than is the calculation of c, which necessitates the squaring of the intercept on the z axis.

It has been shown by Ricci[2] and others that two end-point inflexions in the titration of a weak acid occur only when c is not less than $27K$ or $27K_w/K$. This consideration does not necessarily invalidate the method of interpreting titration curves described above; nevertheless, when K is greater than c in magnitude, it is necessary for h or K_w/h to be in excess of m in order to obtain the useful part of the pH - x curve. This is attended by a considerable error, and in practice it has been found that the treatment is impracticable if the value of c is less than K or K_w/K. A demonstration of this effect is to be found in the pH - x curves for quinine and brucine in Fig. 3, curves C and D. The pK_1 value of quinine, which has been calculated from Fig. 5, curve C, to be 4·13, is of a magnitude that permits the calculation to be made at a concentration of 10^{-3} M. The lower part of the brucine curve in Fig. 3, curve D, shows x almost invariant with pH, and no information on the pK_1 value can be made, apart from the statement that it must be of the order of 2 or less.

As the application of equation (10) demands a measurement of $\triangle h$, it is essential that the pH meter assembly used should be properly calibrated and also adequately stable and sensitive. The titration curves in Fig. 1 and 3 show that the useful pH range of the titration is less than 2 pH units; as it is desirable to take at least 20 measurements within this range, the pH meter should be capable of accurately measuring a pH difference in the region of 0·05 unit.

Careful and patient technical assistance was provided in this investigation by Miss Sylvia Morrissey.

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Integrated-current Source for Automatic Coulometric Titrations*

By L. E. SMYTHE

A simple and inexpensive integrated-current source for automatic coulometric titrations has been developed. The unit is suitable for use with a commercial automatic pH titrimeter and may be used with wide variations in mains voltage. "Fast" or "slow" coulometric titrations may be carried out with a choice of six current ranges (5 to 100 mA) for titrations up to or near to the end-point, and a "slow" 1-mA range for use when approaching the end-point. The "fast" and "slow" outputs from the automatic pH titrimeter provide for automatic changing from fast to slow coulometric titrations and stopping at the selected end-point. The utility of the equipment is demonstrated by the determination of chromium in dichromate solutions by coulometric titration with electro-generated ferrous ions, use being made of potentiometric end-point detection. The unit should prove suitable for most other coulometric titrations and could be adapted for amperometric and spectrophotometric end-point detection.

Coulometric titrations now form a well established section of electro-analysis and the rapidity of development may be gauged from the publication of some eighty papers in the past 2 years. The principles of coulometric analysis and details of earlier and more recent methods are given by Lingane[1] and Delahay,[2] and recent apparatus and methods are reviewed by De Ford.[3]

The coulometric method is of great importance in the field of automatic analysis, principally because of the ease of interpretation and utilisation of electrical and time-based phenomena with which it is concerned. The electronic control equipment may be set up some distance from the coulometric cell, and automatic coulometric titrations are thus well suited for the determination of constituents of certain active solutions. An example is the coulometric titration of chloride and of chromium in homogeneous reactor-type solutions.[4]

A disadvantage of coulometric titration procedures for every-day laboratory use has been the often elaborate and expensive equipment[5,6] required for close control of currents

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of the order 1 to 100 mA to within ± 0·01 per cent. Because of the limitations of the constant-current method, in which the number of coulombs is calculated from the product of current and time, some attention has been devoted to the development of a precise direct-reading coulometer.

Parsons, Seaman and Amick[7] have described a current source in which a low-inertia integrating motor fitted with a counter is used in conjunction with a 70-ohm series resistor immersed in transformer oil. The current source was useful for currents of 150 to 250 mA, and current levels of 12·5 to 13·7 mA were also studied. This circuit does not provide great flexibility as regards choice of current range, and for some current ranges the 24-volt motor is operated at a reduced input voltage owing to the resistor circuitry. There is a slight deviation from linearity of the motor-speed - voltage curve, which becomes greater than 0·5 per cent. when the motor is operated at an input voltage below 5 volts.

The integrated-current source described in this paper obviates the difficulties mentioned above, is versatile and inexpensive and is suitable for operation from a 230 to 240-volt 50 cycles per second a.c. supply.

Experimental

Details of apparatus—

The integrated-current source for automatic coulometric titrations incorporating two low-inertia integrating motors provides the following facilities—

(*a*) Choice of six current ranges of 5, 10, 20, 50, 75 and 100 mA for automatic or manual titrations up to or near to the end-point.

(*b*) A "slow" 1-mA current range for automatic or manual coulometric titrations; used when titrating microgram amounts of the constituent, or when approaching the end-point.

(*c*) Use of the "fast" and "slow" outputs from a commercial automatic pH titrimeter provides for automatic changing from "fast" to "slow" coulometric titrations and stopping at the selected end-point.

(*d*) Selected switching of 100 watt wire-wound resistors operates the integrating motors at ± 10 per cent. of their specified input voltage for all current ranges.

(*e*) A simple d.c. power unit incorporated in the apparatus coupled with the efficient integrating property of the motor makes the equipment suitable for use with variation in the a.c. mains voltage (nominal 230 volts) of ± 20 per cent. over periods up to 10 minutes. Under these conditions the standard deviation for the factor on the 10-mA range was ± 0·03 per cent.

Fig. 1 shows the essential circuitry of the integrated-current-source for automatic coulometric titrations. Fig. 2 shows the complete equipment for automatic coulometric titrations, comprising an integrated-current source, a coulometric cell for the electro-generation of titrant and the automatic pH titrimeter. Magnetically operated stirring is employed with the titration cell.

The six switch positions on the integrated-current source, corresponding to the above-mentioned current ranges, were calibrated in terms of milli-equivalents per count of the "fast" motor. The 1-mA range was also calibrated in terms of milli-equivalents per count of the "slow" motor. The calibrations were carried out by using for current measurement an oil-filled standard 1-ohm resistor (H. Tinsley, type 1659, 3-ampere rating, R = 1·00008 ohm at 20° C) and a high-precision potentiometer (Gambrell, type 12244/1). Counts were recorded (± 0·01 count) for periods of 600 seconds (timed with a calibrated electric permanent magnetic operated stop clock registering to 1/100th second over 12 minutes (Synchromatic Time Recording Co., model 310/0407). Current was measured at 0, 300 and 600 seconds, and averaged. The switch factors were then calculated from the expression—

$$\frac{\text{current (amperes)} \times \text{time (seconds)}}{96{\cdot}492^{*} \times \text{counts}} = \text{milli-equivalents per count.}$$

The cell used for coulometric titrations with electro-generated ferrous ions is essentially that of Cooke and Furman,[9] of capacity 50 ml and a scaled down 10-ml version. Provision was made for either tungsten - platinum or saturated-calomel electrode - platinum potentiometric end-point detection. Although provision was made for titrations in an inert atmosphere

* 96,492 coulombs = 1 chemical equivalent (Craig and Hoffman[8]).

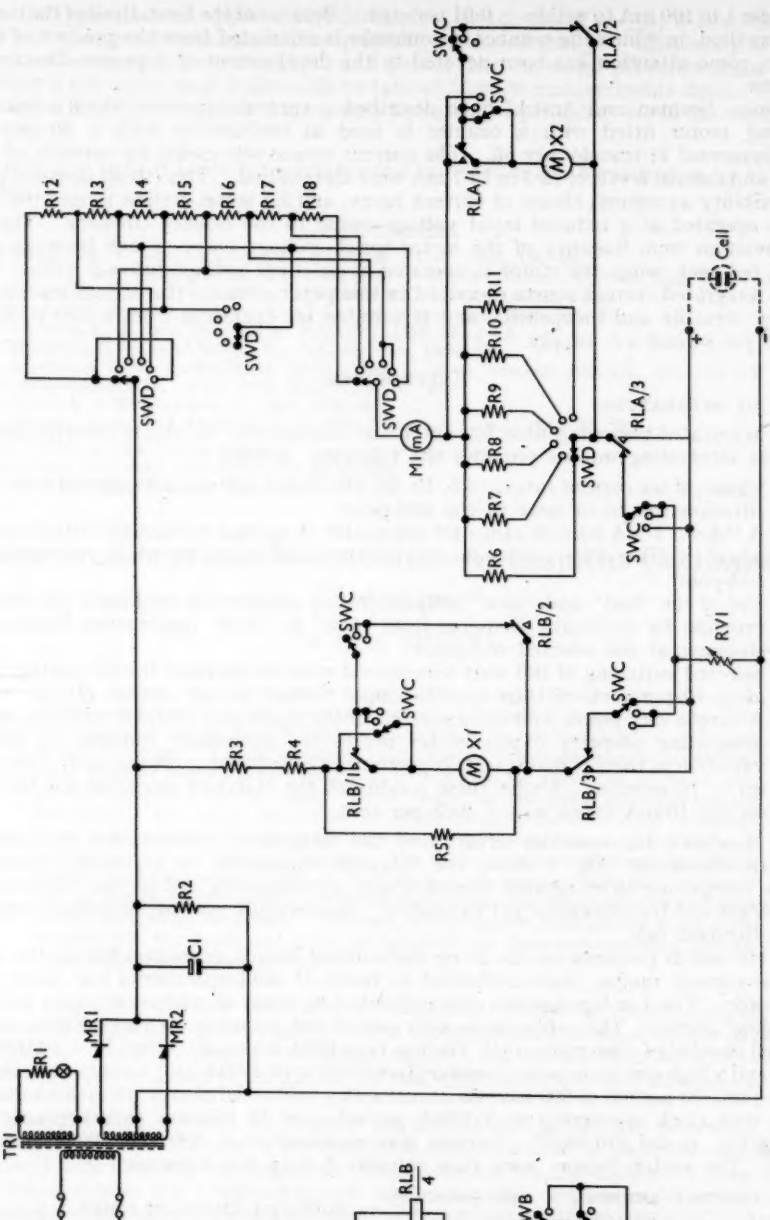

Fig. 1. Integrated-current source for automatic coulometric titrations

(with sub-milligram amounts of the constituent to be determined), it was found that carrying out blank determinations obviated this need. The blank determinations also made allowance for traces of impurities in reagents and any slight positive or negative bias in end-point detection.

The automatic pH titrimeter (model 24) was manufactured by Electronic Instruments Ltd., Richmond, Surrey, and was capable of end-point settings within the ranges pH 3 to 11 and + 400 to − 800 mV. The titrimeter also provided (*i*) two separate input sockets, so that while one titration is being performed another can be set up, and (*ii*) "fast" to "slow" change over within the range 0 to 300 mV (as selected) in advance of the end-point. The titrimeter balance point is sensitive to ± 3 mV and the change in e.m.f. at the end-point, for the determinations studied, was usually 250 to 350 mV during a fraction of a count. The shape of the e.m.f. - count curve[9] for end-point detection should be determined for the particular concentration range of constituent by manual incremental titration with the potentiometric electrode system coupled to the terminals of a direct-reading pH meter. Alternatively, the course of such a titration may be followed by noting the balance point on the titrimeter itself after various increments (counts). Typical titrimeter settings for the titration of 1 mg of chromium in a cell volume of 10 ml were: end-point setting, − 100 mV; function switch set to MV RISING; "fast - slow change-over" setting, 80 mV; tungsten lead connected to glass-electrode terminal; platinum lead connected to reference-electrode terminal. The integrated-current supply was operated with the APPROX. MA switch on 5 mA and the control switch on AUTOMATIC. For the determination of sub-milligram amounts the whole titration should be carried out on the 1-mA range.

AUTOMATIC COULOMETRIC TITRATIONS—

Various trials were carried out in order to demonstrate the utility of the equipment for automatic coulometric titrations. The determination of chromium in standard potassium dichromate solution, by titration with electro-generated ferrous ions, was chosen because potassium dichromate is a reliable primary standard and the volumetric titration with ferrous solutions is long-established. The determination of chromium in steels and cerium in the presence of uranium and lanthanum with electro-generated ferrous ions was tried, and the accuracy of determination was comparable with that for chromium in dichromate.

DETERMINATION OF CHROMIUM IN STANDARD POTASSIUM DICHROMATE SOLUTIONS—

For this determination the following solutions were prepared—

Ferric alum solution—Prepared by dissolving 290 g of analytical-reagent grade ammonium ferric sulphate, $(NH_4)_2SO_4.Fe_2(SO_4)_3.24H_2O$ in 200 ml of distilled water containing 20 ml of concentrated sulphuric acid, sp.gr. 1·84, in a 1-litre beaker. Then 90 ml of concentrated sulphuric acid were added and the solution was diluted to approximately 900 ml; 30 ml of 100-volume hydrogen peroxide were added and the solution was warmed on a hot-plate at 50° to 70° C for ½ hour or until the evolution of oxygen ceased. (This procedure ensured that the solution was free from ferrous ions and was more satisfactory than that described by Cooke and Furman.[9]) When the solution had cooled, it was passed through a sintered filter and diluted to 1 litre.

Standard dichromate solutions—Three 0·1000 *N* standard potassium dichromate solutions were prepared for cross-checking in the trials of the integrated-current supply. Analytical-reagent grade potassium dichromate was recrystallised and, after a preliminary drying, the crystals were ground to a fine powder in an agate mortar and dried at 140° to 150° C to constant weight. The required weight was then used to prepare the dichromate solutions. Dilution of the 0·1 *N* solutions was used for the preparation of 0·01 *N* and more dilute solutions.

The procedure is as follows—

By pipette place the solution to be analysed into the coulometric titration cell. Add 2 ml of 18 *N* sulphuric acid and then 5 to 30 ml of the ferric alum solution. Use about 5 ml of the ferric alum solution for sub-milligram amounts of chromium (1 or 5-mA current ranges), 10 ml for current ranges of 10 to 50 mA and 20 to 30 ml for current ranges of 70 to 100 mA, depending on the chromium content of the solution. Dilute the solution sufficiently to cover the electrodes after inserting a polythene-covered stirrer. To carry out the titration, set the titrimeter as previously described, record the "slow" and "fast" motor readings and turn on the titrimeter AUTOMATIC switch. On completing the titration, again record the motor

readings. The titrimeter and integrated-current supply should be switched on 10 minutes before the titration is commenced and set on the instrument the required current range. This permits the resistors to attain normal working temperature.

The results are shown in Table I.

TABLE I

DETERMINATION OF CHROMIUM IN POTASSIUM DICHROMATE

Cell volume, ml	Chromium taken, mg	Chromium found (average), mg	No. of determinations	Relative mean error, %
40	17·34	17·52	4	−0·2
10	3·47	3·46	7	−0·3
10	0·1734	0·1726	4	−0·5
10	0·01734	0·0183	5	+5·0

CONCLUSIONS

The results show that good accuracy can be attained with the integrated-current source for automatic coulometric titrations, in the milligram and sub-milligram range down to a cell volume of 10 ml. The methods offer advantages in the automatic determination of elements in active solutions.[4] From a consideration of this work and other work with micro-coulometric equipment,[10,11,12] the accuracy of the method (with concentrations below 0·1 mg of the element in a cell volume of 10 ml) should be capable of further improvement, by using suitable micro-cells with provision for de-gassing. The equipment described should be suitable for the majority of coulometric titrations.

The assistance of J. C. Boag with circuit design is gratefully acknowledged.

APPENDIX

LIST OF COMPONENTS USED IN THE CONSTRUCTION OF THE INTEGRATED-CURRENT SOURCE FOR AUTOMATIC COULOMETRIC TITRATIONS

(Fig. 1)

R_1 = 10-ohm, 1·5-watt, wire-wound vitreous-enamelled resistance.
R_2 = 33,000-ohm, 10-watt, wire-wound vitreous-enamelled resistance.
R_3, R_4 = 150,000-ohm, 1-watt, composition non-insulated resistance.
R_5 = 24,000-ohm, 1-watt, composition non-insulated resistance.
R_6 = 4700-ohm, 100-watt, wire-wound vitreous-enamelled resistance.
R_7, R_{15} = 2200-ohm, 100-watt, wire-wound vitreous-enamelled resistance.
R_8, R_{13} = 1200-ohm, 100-watt, wire-wound vitreous-enamelled resistance.
R_9 = 470-ohm, 100-watt, wire-wound vitreous-enamelled resistance.
R_{10} = 330-ohm, 100-watt, wire-wound vitreous-enamelled resistance.
R_{11} = 220-ohm, 100-watt, wire-wound vitreous-enamelled resistance.
R_{12} = 1500-ohm, 100-watt, wire-wound vitreous-enamelled resistance.
R_{14} = 3300-ohm, 100-watt, wire-wound vitreous-enamelled resistance.
R_{16} = 12,000-ohm, 100-watt, wire-wound vitreous-enamelled resistance.
R_{17} = 15,000-ohm, 100-watt, wire-wound vitreous-enamelled resistance.
R_{18} = 20,000-ohm, 100-watt, wire-wound vitreous-enamelled resistance.
C_1 = 8-μF, 600-V, fixed paper - foil capacitor.
RV1 = 1000-ohm, 20-watt, variable wire-wound linear resistance.
RLA = 500-ohm coil, K3000-type, magnetic relay (4 contacts, 2 of which are platinum; only 3 are used).
RLB = 500-ohm coil, K300-type, magnetic relay (4 contacts, 2 of which are platinum).
MR1, MR2 = Metal rectifier (S.T.C. code MDA25-20-1GZ).
TR1 = Power transformer (Gardner's Radio type to AERA specification No. 182).
M1 = 150-mA, 2½-inch, flush-type moving-coil, d.c. ammeter (Sangamo Weston Ltd.).
X1, X2 = 24-V, d.c., low-inertia integrating-type motor (Electro Methods type 913).
PLA = Three-pole, position 5, fixed plug.
PLB = Six-pole, position O, fixed plug.
PLC = Tow-pole, position O, fixed plug.
SWA = 3-amp., double-pole change-over toggle switch (used as double-pole ON - OFF).
SWB = 3-amp., double-pole change-over toggle switch.
SWC = Six-pole, three-way, non-shorting, rotary wafer switch.
SWD = Four-pole, six-way, shorting, rotary wafer switch.

Fig. 2. Equipment for automatic coulometric titrations

[To face p. 232

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September 20th, 1956

A Method for the Determination of Acetic Anhydride in Mixtures with Acetic Acid*

BY T. ELLERINGTON AND J. J. NICHOLLS

The titration of amines in acetic acid with perchloric acid, with either a potentiometric or visual end-point, is applied to the determination of acetic anhydride in acetic anhydride - acetic acid mixtures. The end-points and accuracy are good.

MANY methods for the determination of acetic anhydride in mixtures of acetic anhydride and acetic acid are modifications of the British Standard method.[1] Such methods are somewhat tedious and suffer from the disadvantages that a small titration error leads to a comparatively large error in the final result. A more recent method involves reaction of the anhydride with an excess of morpholine and titration of the excess of reagent with a solution of hydrochloric acid in methanol.[2]

In the method described in this paper the acetic anhydride is allowed to react with excess of standard aniline solution in glacial acetic acid, and the excess of aniline is titrated with perchloric acid in acetic acid.[3,4] The end-point may be determined either potentiometrically with use of a glass indicator electrode and a saturated-calomel reference electrode in conjunction with a high-impedance millivoltmeter or visually by using a suitable indicator (see p. 235). A closed potentiometric titration cell incorporating agitation with dry nitrogen and fitted with a trap for acetic acid vapour is shown in Fig. 1.

All the work to be described involves reactions in non-aqueous solution, and hence introduction of water into the titration cell is to be avoided as far as possible. It should also be noted that a glass electrode immersed in glacial acetic acid may assume a positive potential with respect to a calomel electrode, and that, if pH readings are taken, the values obtained are in arbitrary units, since pH has no significance in non-aqueous solution.

Experimental

Preparation and standardisation of reagents—

Sodium carbonate in glacial acetic acid, 0·1 N—Prepared by drying analytical-reagent grade anhydrous sodium carbonate at 270° ± 10° C to constant weight, dissolving 2·6501 g of the solid in glacial acetic acid and making up to 500 ml in a calibrated flask.

Perchloric acid in glacial acetic acid, 0·1 N—Anhydrous perchloric acid is difficult to obtain, and the acid is usually available as an approximately 70 per cent. solution having a specific gravity of 1·7. The reagent is prepared by diluting 9·5 ml of the 70 per cent. acid

* Presented at the XVth International Congress on Pure and Applied Chemistry (Analytical Chemistry), Lisbon, September 8th to 16th, 1956.

to 1 litre with glacial acetic acid. This solution is standardised against the 0·1 *N* sodium carbonate in glacial acetic acid, the standard actually being sodium acetate, which functions as a base of comparable strength to aniline (see Fig. 2) under the conditions of the experiment. Potassium hydrogen phthalate can also be used as a standard. The reagent solution is not stable and requires re-standardising at least every 3 days.

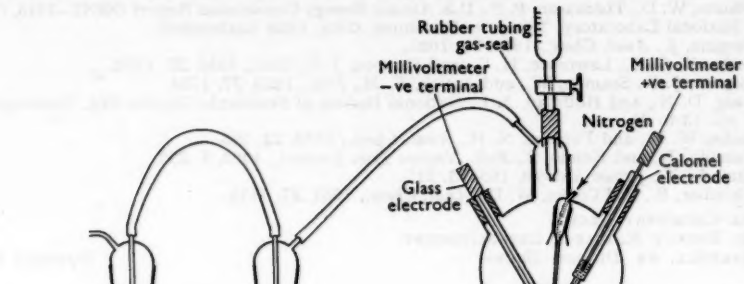

Fig. 1. Potentiometric-titration apparatus, with a trap for acetic acid vapour

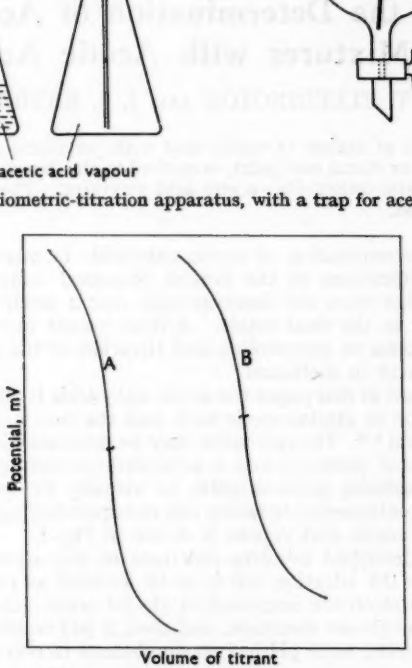

Fig. 2. Standardisation curves: curve A, perchloric acid against sodium carbonate; curve B, aniline against perchloric acid

Aniline in glacial acetic acid, 0·1 N—Prepared by diluting 9·1 ml of redistilled aniline to 1 litre with glacial acetic acid. This solution is standardised against the 0·1 *N* perchloric acid by potentiometric titration; it is unstable and requires standardising every day.

DETERMINATION OF ACETIC ANHYDRIDE—

In the method to be described acetic anhydride is allowed to react with excess of aniline until quantitative reaction has occurred, and the remaining aniline is then titrated with perchloric acid.

The development of the method was effected in three main stages, namely—

(*a*) a series of assays of pure acetic anhydride to establish the best technique,
(*b*) a series of experiments to determine the time required for the reaction to go to completion, and
(*c*) a series of determinations of acetic anhydride in mixtures of anhydride and acid of known composition.

Development of technique—In the development of the technique of introducing the reactants to each other, three methods were tried, the third being successful.

In the first, the anhydride was introduced into the aniline solution, contained in a stoppered flask, by means of a Lunge - Ray pipette. There was a tendency for the tip of the pipette to catch on the ground neck of the flask during this procedure and deposit a small amount of anhydride, which did not enter into reaction. Low results were, therefore, sometimes obtained.

In the second method, the stoppered flask containing the standard aniline solution was weighed before and after introduction of the anhydride. There was a tendency for acetic acid vapours to "creep" between the ground-glass surfaces, so that, when the stopper was removed to allow the introduction of the sample, some acid evaporated, so giving a low apparent weight of sample, which led to high results.

In the third, successful method, the anhydride was weighed directly into a clean dry calibrated flask and diluted to the mark with glacial acetic acid. A suitable aliquot was then put by pipette into the standard aniline solution. This method of introducing the reactants to each other consistently gave the expected results.

Determination of time required for reaction—A 1·1350-g amount of pure acetic anhydride (99·9 per cent. by the British Standard method[1]) was weighed into a clean dry 100-ml calibrated flask and diluted to the mark with glacial acetic acid.

Into each of four stoppered clean dry 150-ml flasks were put by pipette 50·0 ml of standardised 0·1 *N* aniline solution and 20·0 ml of the anhydride solution. The last mixture to be prepared was titrated as soon as possible after the first mixing, even so 3 minutes had elapsed, and the others at longer intervals. The recoveries of acetic anhydride in these four experiments, which may be taken as the percentages of reaction having occurred, were plotted against the time required, as shown in Fig. 3.

Determination of acetic anhydride in mixtures of known composition—Four mixtures of acetic anhydride and acetic acid, together with a "pure" anhydride, were assayed by the method described. The anhydride gave a result of 96·9 per cent. w/w by reaction with aniline and titration with perchloric acid. The four mixtures were made up accurately by weight.

The acetic anhydride contents of the mixtures, based on both chemical and potentiometric assays of the pure anhydride used, are shown in Table I, together with the results by potentiometric titration and with use of a visual indicator.

Method

Into a clean and thoroughly dry 100-ml calibrated flask weigh accurately sufficient sample to contain approximately 1 g of acetic anhydride and dilute to the mark with glacial acetic acid. By pipette put into a 150-ml stoppered flask 50·0 ml of standardised aniline in acetic acid solution. Add by pipette 20·0 ml of the prepared solution of the sample in acetic acid, set aside for a minimum of 40 minutes, and transfer the contents of the flask quantitatively to the titration vessel with the aid of glacial acetic acid; titrate the excess of aniline with standard perchloric acid in glacial acetic acid solution.

$$\text{Acetic anhydride, per cent.} = \frac{(B - T) \times 0{\cdot}01021 \times F \times 100}{0{\cdot}2 \times W},$$

where B = volume in ml of standard perchloric acid solution equivalent to 50·0 ml of aniline in acetic acid solution,
T = titre in ml of perchloric acid solution after reaction,
F = factor of 0·1 *N* perchloric acid solution, and
W = weight of sample taken.

Use of an indicator for a visual end-point—

Wagner, Brown and Peters[3] refer to crystal violet as being a suitable indicator for titrations, giving a good potentiometric-titration curve. This indicator has been tried and,

once the operator had become accustomed to the rather subtle colour change at the end-point, the results agreed with those found by plotting the titration curve. When added to the amine in acetic acid, the indicator was violet and on titration with perchloric acid underwent the following colour changes—

violet → blue-violet → blue → green-blue → blue-green → "pure" green → yellow-green → green-yellow → yellow.

TABLE I

DETERMINATION OF ACETIC ANHYDRIDE IN MIXTURES

Acetic anhydride present in mixture, calculated—		Acetic anhydride in mixture, determined with a—	
from a chemical analysis, %	from a potentiometric determination, %	potentiometric end-point, %	visual end-point, %
96·9	96·2	96·2	96·2
73·7	73·2	73·2	72·8
48·8	48·5	48·8	48·5
25·1	24·9	25·3	25·3
1·12	1·11	1·18	1·18

The colour indicative of the correct end-point varies with the substance being titrated, and it is advisable to mark the colour changes on an actual titration curve for any given titration to determine the correct visual end-point. The "pure" green was the correct visual end-point colour for aniline.

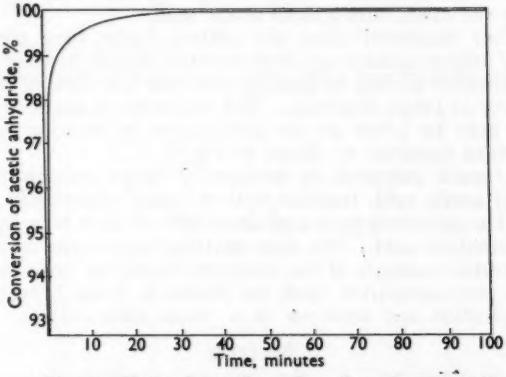

Fig. 3. Rate of reaction of acetic anhydride with excess of aniline at 20°C

The colour changes of the indicator were observed in various aqueous solutions by adjusting the pH by suitable addition of acid or alkali, in the hope of finding a solution that, on being added, would give the "pure" green colour of the indicator. Such a solution could be used for matching when the visual method of detecting the end-point is used. It was found that the colour of the indicator depended not only on the pH of the solution but also on its composition. Finally, however, an acetic acid - acetate buffer solution was selected, the pH being adjusted by means of 10·0 *N* hydrochloric acid. The colours of the indicator and the pH of these solutions are shown in Fig. 4.

A suitable indicator solution is prepared by dissolving 0·1 g of crystal violet in 100 ml of glacial acetic acid; 0·1 ml of indicator solution is used for every 50 ml of solution.

Preparation of colour standards—Three colour standards were developed to provide matching to the indicator colour (*a*) just before the end-point, (*b*) at the end-point, and (*c*) just after the end-point. These are prepared by adding indicator solution (0·1 ml per 50 ml) to the appropriate buffer solution immediately before required. The buffer solutions are made by mixing 50·0 ml of *N* sodium acetate solution, 10·0 ml of 99 to 100 per cent. acetic acid and various amounts of 10·0 *N* hydrochloric acid, and then diluting to 250 ml

with water. For: (*a*) buffer solution of pH 0·7, blue-green with indicator added, 8·9 ml of 10·0 *N* hydrochloric acid are required; (*b*) buffer solution of pH 0·6, "pure" green with indicator added, 10·5 ml of 10·0 *N* hydrochloric acid are required; (*c*) buffer solution of pH 0·5, yellow-green with indicator added, 12·5 ml of 10·0 *N* hydrochloric acid are required.

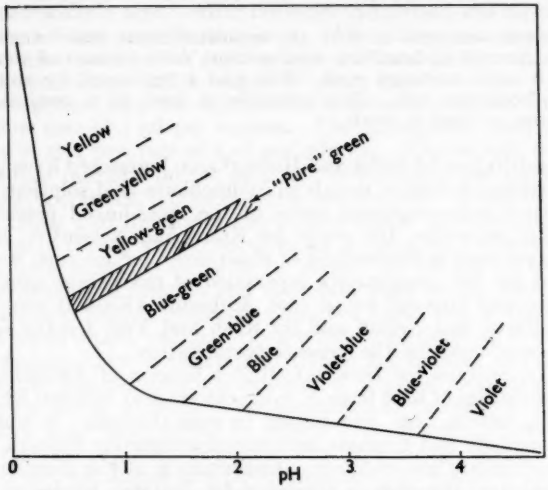

Fig. 4. Colour changes of crystal violet in acetic acid - acetate buffer solutions; sharp changes are indicated by full lines and more gradual changes by broken lines

We are indebted to British Industrial Solvents (a Division of the Distillers Company Limited), in whose laboratories this work was carried out, for permission to publish this paper.

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British Industrial Solvents
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August 2nd, 1956

The Use of an Anion-exchange Resin in the Determination of Traces of Lead in Food

By E. I. JOHNSON and R. D. A. POLHILL

Microgram amounts of lead are separated from most other ions by absorption from *N* hydrochloric acid solution on a column of the chloride form of an anion-exchange resin. The lead is recovered by elution with 0·01 *N* hydrochloric acid. This principle is used in a method for the determination of lead in foods.

Recently Kraus and Nelson,[1,2] Miller and Hunter[3] and Jentzsch[4,5] have published information about the behaviour of various metals in hydrochloric acid solutions with the chloride form of strongly basic anion-exchange resins of the cross-linked polystyrene quaternary ammonium type. In particular, the paper by Kraus and Nelson[2] on lead and bismuth, together with our experience of the method of Rush and Yoe[6] for zinc, suggested that these resins could be used for the quantitative separation of microgram amounts of lead from acid solution. Miller and Hunter[3] found that Amberlite IRA-400 was as satisfactory as Dowex 1 (used by Kraus and Nelson and by Rush and Yoe) for the separation of zinc. Amberlite IRA-400 was used for the present investigation.

It was found, as reported for Dowex 1, that a column of Amberlite IRA-400 would absorb microgram quantities of lead from *N* hydrochloric acid solution while allowing alkali metals, alkaline-earth metals, iron, and copper to pass through. It was also found that small amounts of phosphate and sulphate ions passed completely through the column when introduced in *N* hydrochloric acid solution. Examination of the distribution curves given by Nelson and Kraus and the elution constants by Jentzsch suggested that very dilute hydrochloric acid could be used to elute the lead. It was found that 0·01 *N* hydrochloric acid quickly eluted the whole of the lead present. It was found possible to adjust the conditions of elution so that the normality of the eluate containing the lead was very close to the normality of a 1 per cent. nitric acid solution. The lead in the eluate could then be easily and conveniently determined by the mono-colour method of Snyder.[7] Further work was directed to the development of a method for the determination of lead in foods and similar materials.

The possibility of interferences with such a method for lead caused us to investigate the behaviour of zinc, bismuth, cadmium, tin and thallium under the conditions of the method. Table I shows the effect in terms of apparent lead of various amounts of these metals in *N* hydrochloric acid solution, placed on the column and subsequently treated as in a determination of lead.

Table I

Effect of various metals on lead determination

Metal	Amount added, μg	Apparent lead found, μg
Tin	2000	Nil
Zinc	500	Nil
Cadmium	200	0·9
Bismuth	230	0·5
Thallium	300	0·7

Tin and thallium pass through the resin with the *N* hydrochloric acid. Bismuth is retained and not eluted. Zinc is retained and eluted with 0·01 *N* acid. Cadmium is retained and eluted much more slowly than zinc. The conditions of the dithizone procedure substantially suppress interference from zinc and cadmium if present in amounts similar to those in Table I.

Experimental

Amberlite IRA-400 anion-exchange resin (analytical grade) purchased in the hydroxyl form was treated by the method of Miller and Hunter.[3] Six grams were reduced by being ground in a mortar until the resin all passed through a No. 60 B.S. sieve. Fines were removed by a No. 100 B.S. sieve and the fraction not passing that sieve was used to make the column.

The resin was soaked overnight in 2 *N* hydrochloric acid. Fines were removed by decantation and the remainder was transferred to the ion-exchange tube, which consisted of a glass tube 15 cm long and 8 mm in internal diameter. The bottom was fitted with a tap to control the flow rate and the top was sealed to a piece of wider tubing to give a reservoir of capacity about 30 ml. A 5-mm plug of cotton-wool was placed in the bottom of the tube and the resin washed in with *N* hydrochloric acid. After the resin had settled, the top of the resin column was held in place with a 2-mm plug of cotton-wool. The column was thoroughly washed with *N* hydrochloric acid, then with 0·01 *N* hydrochloric acid and finally with 10 ml of *N* hydrochloric acid. The resin column had a length of about 8 cm.

Substances were passed into the column in the form of solutions in 5 ml of *N* hydrochloric acid, at a flow rate of 1 ml per minute. The column was then washed with 30 ml of *N* hydrochloric acid at a flow rate of 2 ml per minute. Elution with 0·01 *N* hydrochloric acid was done at a flow rate of 1 ml per minute. The column was allowed to drain under gravity between these operations, but no pressure was applied. It was found that on elution with 0·01 *N* hydrochloric acid the first 2·5 to 3 ml of eluate were undiluted *N* hydrochloric acid. The next 10 ml of eluate, which contained all the lead, had an acidity of about 0·2 *N*.

Solutions containing elements likely to be present in the ash of foods were prepared in 5 ml of *N* hydrochloric acid solution and their behaviour on the resin column when subjected to the washing and elution procedure described above was studied. These solutions contained up to 1·5 mg of copper and iron, up to 0·25 g of calcium, up to 0·15 g of magnesium and up to 0·15 g of phosphorus as phosphate. All of the calcium and magnesium and the bulk of the iron, copper and phosphate were found in the first 15 ml of *N* hydrochloric acid wash. The remainder of the phosphate was contained in the next 5 ml. The last portion of the *N* hydrochloric acid wash contained only a trace of iron and copper.

It was found that the retention of lead by the column was reduced by the presence of large amounts of calcium and magnesium phosphates, but that amounts up to 0·1 g of calcium, magnesium or phosphorus as phosphate did not effect retention or recovery of lead. Sulphate behaved similarly to phosphate. Small amounts such as result from the ashing of 5 g of food have no effect on retention of lead by the column, but large amounts such as would remain from the wet or sulphated ashing of a food temporarily destroy the capacity of the column to retain lead and most other metallic ions. The passing of 50 ml of *N* sulphuric acid through the column was in fact found to be a convenient method of clearing it from accumulations of ions normally retained and not eluted, *e.g.*, bismuth and cadmium. The subsequent passage of 30 ml of *N* hydrochloric acid restored the column to the chloride form.

Amounts of lead up to 40 μg in 2 ml of *N* hydrochloric acid were run on to the column, which was then washed with 30 ml of *N* hydrochloric acid. All the lead was recovered in the first 10 ml of eluate with 0·01 *N* hydrochloric acid, after rejection of the 2·5 ml of undiluted *N* hydrochloric acid that preceded it. Second and third 10-ml portions of eluate were found to be free from lead.

We are of the opinion that this resin column made and used in the way we have described is a useful and convenient device for freeing a hydrochloric acid solution containing lead in microgram quantities from substances that would otherwise interfere with the determination of the lead. It has certain limitations besides those we have already indicated. We did not find it possible to use it as a simple means of concentrating quantitatively very dilute solutions of lead. Washing the column with volumes of *N* hydrochloric acid greatly in excess of the 30 ml normally used caused the lead to move down and ultimately off the column despite the maintenance of the concentration of the acid. This has been taken into consideration in the design of the following method for the determination of lead in foods. The method has the merit of requiring fewer and less reagents than any other of which we are aware. As a consequence of this, the method gives lower blanks than other methods in which reagents of the same "lead-free" standard are used. The lowness of this blank is not due to loss of lead in the *N* hydrochloric acid wash. This possibility was carefully investigated and no lead was found in the 35 ml of acid emerging from a column that contained 40 μg of lead. The method used would have detected 0·2 μg of lead.

Method

Reagents—

Amberlite IRA-400 anion-exchange resin.
Hydrochloric acid, 2 N, N *and* 0·01 N.

Chloroform.

Dithizone stock solution—A 0·1 per cent. w/v solution of dithizone in chloroform.

Dithizone working solution—Shake 6 ml of the stock dithizone solution with 10 ml of 0·5 *N* ammonium hydroxide solution and reject the chloroform layer.

Solution A—Mix 340 ml of ammonium hydroxide, sp.gr. 0·880, 75 ml of 2 per cent. w/v sodium sulphite, Na_2SO_3, solution, 30 ml of 10 per cent. w/v potassium cyanide solution and 605 ml of water.

Standard lead solution—(*a*) Dissolve 1·60 g of lead nitrate in water, add 10 ml of concentrated nitric acid and dilute to 1 litre.

(*b*) Dilute 1 volume of (*a*) to 100 volumes with water. Prepare dilution (*b*) freshly as required.

1 ml ≡ 10 μg of lead.

Magnesium nitrate solution—A 10 per cent. w/v solution of magnesium nitrate, $Mg(NO_3)_2.6H_2O$, in water.

Further details about the preparation in a lead-free state, storage and standards required of these reagents are given elsewhere.[8,9]

APPARATUS—

An ion-exchange tube—The design and filling of this tube with resin have already been described under "Experimental".

PROCEDURE FOR DESTROYING ORGANIC MATTER AND SEGREGATION OF LEAD—

Ash a suitable quantity of sample, containing not more than 5 g of dry matter, in a silica or platinum dish at a temperature not exceeding 500° C. If the material is otherwise difficult to ash, up to 5 ml of magnesium nitrate solution may be added as an ashing-aid. Dissolve the ash in 4 ml of 2 *N* hydrochloric acid, cover the dish with a watch-glass and heat it on a steam-bath for 10 minutes. Filter through a cotton-wool plug held in the stem of a small conical funnel into a 10-ml measuring cylinder, washing the dish and filter with a few millilitres of water. Volumes must be kept low at this stage, the total of filtrate and washings not exceeding 8 ml, and the exact volume should be noted. To the resin column prepared as previously described and wet with *N* hydrochloric acid, add a 5-ml aliquot of the ash solution. The 5 ml should not contain more than 40 μg of lead. Adjust the flow rate to 1 ml per minute. When all the 5 ml of solution is below the level of the top of the resin, add 25 ml of *N* hydrochloric acid and allow it to pass through the column at the rate of 2 ml per minute. Elute the lead from the column with 0·01 *N* hydrochloric acid at a flow rate of 1 ml per minute, rejecting the first 2·5 ml, which should be undiluted *N* hydrochloric acid free from lead, and collecting the next 10 ml.

PROCEDURE FOR DETERMINING LEAD—

To the eluate in a 100-ml separating funnel add 30 ml of solution A, exactly 10 ml of chloroform and 0·5 ml of the dithizone working solution; shake vigorously for 1 minute and allow to settle. Run off a little of the chloroform layer. Insert a plug of cotton-wool into the dry stem of the funnel and, after rejecting the first runnings, fill a 1-cm spectrophotometer cell with the chloroform solution. Measure the optical density against chloroform at 520 mμ.

Prepare a blank solution under the same conditions as the test, omitting only the sample, and determine the optical density. To prepare a calibration graph measure 0, 1·0, 2·0, 3·0 and 4·0 ml of standard lead solution into separating funnels containing 2 ml of *N* hydrochloric acid and add water to give a total volume of 10 ml in each funnel. Proceed as described above.

As the method is sensitive, all the precautions usual in this type of work must be observed.[8,9] Correct the observed result for the reduction due to the taking of part only of the ash solution for the final determination.

RESULTS

Typical results for lead found in various samples are shown in Table II.

Blanks were usually found to be less than 1 μg of lead and were constant for one set of reagents. The resin column may be re-used repeatedly after regeneration with *N* hydrochloric acid. Accumulations of ions not readily eluted with hydrochloric acid can be removed

by running 50 ml of *N* sulphuric acid through the column, followed by 30 ml of *N* hydrochloric acid to regenerate the chloride form.

TABLE II

DETERMINATION OF LEAD

Sample	Lead by usual laboratory method[9]	Lead by proposed method
15 μg of lead	—	15·5 μg
40 μg of lead	—	39·9 μg
5 g of cocoa	0·84 p.p.m.	0·82, 0·84 p.p.m.
5 g of syrup	5·6 p.p.m.	5·3 p.p.m.
5 g of curry powder	4·3 p.p.m.	4·3 p.p.m.
6·5 g of cocoa + 30 μg lead (calculated 5·4 p.p.m.) ..	—	5·4 p.p.m.

We express our thanks to the Government Chemist for permission to publish this paper.

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November 5th, 1956

Flame-photometric Determination of Magnesium in Plant Material

A Study of the Emission of Magnesium in a Highly Reducing Oxygen - Acetylene Flame

BY KARIN E. KNUTSON

The sensitivity obtained with the 2852 A line emitted by the magnesium atom was considerably greater with a highly reducing carburising flame than with a neutral one. Examination of the sources of error showed that phosphate and sulphate ions gave rise to negative interference, which could be largely eliminated by the presence of an excess of calcium ions. The accuracy of the method was checked by analysis of prepared samples of known magnesium content and by comparative chemical and flame-spectrophotometric analysis of plant material. In the study of the precision the standard deviation of a single determination was less than 1 per cent. The "detection limit" was 0·06 p.p.m.

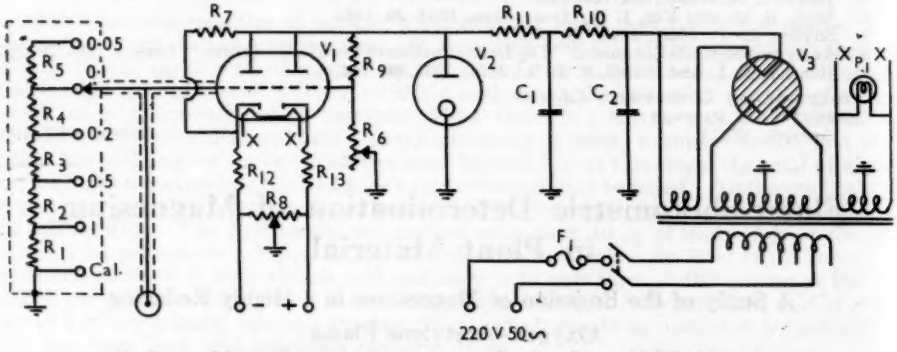
THE chemical determination of magnesium has always been an involved and time-consuming procedure when applied to samples of complex composition, such as plant and soil materials. Accordingly, methods for the flame-photometric and spectrophotometric determination of magnesium have been evolved. One difficulty of these methods lies in the flames used; for instance, acetylene - air, propane - oxygen - air and acetylene - oxygen mixtures themselves produce a high emission at the utilisable wavelengths: 2851, 3721, 3811 and 3850 A. Hence, there is insufficient contrast between the magnesium emission and the background radiation, and it is difficult to determine small quantities of the element.[1,2,3,4,5,6] Good results have, however, been achieved, although mostly with relatively high magnesium

concentrations.[7,8,9,10] There are many reports in the literature on the disturbing effect of other ions, such as those occurring in plant samples.[3,4,8,9,11,12] Consequently, in the work described in the paper, attempts have been made to increase the sensitivy of the method. Attention has also been given to the significance of the presence of other ions.

Experimental

Apparatus—

Preliminary experiments with a Beckman DU spectrophotometer with attachment for a propane - oxygen - air flame showed that its sensitivity was unsatisfactory. Moreover, it is inconvenient to work with a compensation instrument in flame-photometric studies. For these experiments, therefore, the equipment consisted of a monochromator with a quartz prism, a burner housing with an atomiser burner for an oxygen - acetylene mixture and regulators (Beckman DU flame spectrophotometer with attachment[13]). In addition, a photomultiplier tube, RCA 1P28, was used with a power supply of 860 volts from dry batteries. There should be means of measuring the current strength or, if a high resistance is introduced, the voltage drop in the anode circuit of the photomultiplier tube. However, as it is necessary to operate the recorder from a low-impedance source, a cathode follower was inserted in the circuit. The circuit arrangement is shown in Fig. 1. The output signal level was adjusted by changing the resistance (resistors R_1 to R_5) across which the input voltage was developed (when a current flows in the phototube). A setting of 0·2 was used for all measurements. The dark-current could be compensated for by means of a zero adjustment control.



R_1, R_2	= 0·9-megohm resistance	R_{11}, R_{12}, R_{13}	= 10,000-ohm resistance
R_3	= 2·7-megohm resistance	C_1, C_2	= 8-μF condensers
R_4	= 4·5-megohm resistance	V_1	= 5692 valve
R_5	= 9-megohm resistance	V_2	= 0C3 (VR 105) valve
R_6	= 500-ohm resistance (ZERO ADJUSTMENT CONTROL)	V_3	= 5Y3GT valve (anode to cathode plate voltage per plate (r.m.s.), 300 volts)
R_7	= 470,000-ohm resistance		
R_8	= 300-ohm resistance		
R_9	= 50,000-ohm resistance	F_1	= 0·8-amp. fuse
R_{10}	= 25,000-ohm resistance	P_1	= 0·2-amp. 6·5-volt bulb

Fig. 1. Circuit diagram of cathode follower for Speedomax recorder, type G

The voltage was measured by means of a Speedomax automatically recording potentiometer, type G, model S, obtained from Leeds & Northrup Co., with a 2·2-second full-scale pen travel. The scale was calibrated in millivolts and the full-scale deflection was 10 mV (240 mm). A valuable advantage of the automatically recording potentiometer is that any error, such as that incurred by a tendency for the atomising efficiency to decrease, is immediately observed. In many measurements, however, the instrument was used without inserting the recording device.

Properties of the flame and their influence on the magnesium emission—

The type of flame is dependent on the volumetric proportions of the oxygen and acetylene supplied to the burner. In a neutral flame the acetylene content is about 48 per cent. and in a carburising flame about 55 per cent.[14] The neutral flame contains carbon monoxide

and hydrogen, and the more strongly reducing carburising flame also contains carbon, which, on account of the high temperature, emits an intensive yellowish white light. With the more strongly reducing carburising flame the ratio of the intensities of the 2852 A line emitted by the neutral magnesium atom and the background radiation is appreciably increased (see Table I). The higher the carburising zone the higher the potentiometer reading for magnesium. The background radiation increases with the proportion of acetylene. The background reading is kept constant by reduction of the slit width.

TABLE I

EMISSION FROM THE NEUTRAL MAGNESIUM ATOM AT 2852 A FOR VARIOUS PARTS OF THE FLAME AND HEIGHTS OF THE CARBURISING ZONE

For all determinations the reading for background radiation was adjusted to 4 mV by means of the monochromator slit. This value has been subtracted from the figures given in the "Potentiometer reading for 10 p.p.m. of magnesium" column. Adjustment was made for dark-current

Burner rating, lb per sq. inch	Slit width, mm	Oxygen pressure, lb per sq. inch	Acetylene pressure, lb per sq. inch	Height of carburising zone above burner tip, mm	Height of exposed part of flame above burner tip, mm	Potentiometer reading for 10 p.p.m. of magnesium, mV
19	0·036	9	1·8	0 to 10	11 to 43	1·3
19	0·025	9	3	0 to 25	11 to 43	2·4
19	0·023	9	5	0 to 45	11 to 43	3·8
19	0·037	9	1·8	0 to 10	11 to 25	2·2
19	0·029	9	3	0 to 25	11 to 25	3·2
19	0·030	9	5	0 to 45	11 to 25	4·4
19	0·049	9	1·8	0 to 10	19 to 34	1·6
19	0·032	9	3	0 to 25	19 to 34	3·6
19	0·030	9	5	0 to 45	19 to 34	5·1
19	0·044	9	1·8	0 to 10	23 to 38	1·9
19	0·030	9	3	0 to 25	23 to 38	2·9
19	0·028	9	5	0 to 45	23 to 38	4·1
10	0·020	7	5	0 to 15	11 to 43	2·1
10	0·045	7	2	0	11 to 25	0·9
10	0·023	7	5	0 to 15	11 to 25	2·6
10	0·021	7	7	0 to 20	11 to 25	3·5
10	0·021	5	5.	0 to 20	11 to 25	2·7
14	0·019	5·5	5	0 to 30	11 to 43	2·4
14	0·026	5·5	5	0 to 30	11 to 25	3·6
14	0·031	5·5	5	0 to 30	23 to 38	4·6

TABLE II

EMISSION FROM THE MAGNESIUM ATOM AT 2852 A AND FROM MAGNESIUM OXIDE AT 3721, 3811 AND 3850 A IN FLAMES OF VARIOUS REDUCING STRENGTHS

The figures given in the "Potentiometer reading for background" column have been subtracted from the observed potentiometer readings to give the figures in the "Potentiometer reading for 100 p.p.m. of magnesium" column. Adjustment was made for dark-current. For all determinations a burner with a rating of 19 lb per sq. inch was used and the height of the exposed part of the flame was 11 to 25 mm above the burner tip

Wave-length, A	Slit width, mm	Oxygen pressure, lb per sq. inch	Acetylene pressure, lb per sq. inch	Height of carburising zone above burner tip, mm	Potentiometer reading for background, mV	Potentiometer, reading for 100 p.p.m. of magnesium, mV
2852	0·034	19	3	0	1·5	3·1
2852	0·020	9	5	0 to 45	1·5	7·4
3721	0·029	19	3	0	1·5	0·5
3721	0·015	9	5	0 to 45	1·5	0·4
3721	0·051	19	3	0	6·0	2·1
3721	0·027	9	5	0 to 45	6·0	0·9
3811	0·052	19	3	0	6·0	1·3
3811	0·025	9	5	0 to 45	6·0	0·5
3850	0·052	19	3	0	6·0	2·0
3850	0·024	9	5	0 to 45	6·0	0·8

The sensitivity for magnesium at the 2852 A line emitted by the neutral magnesium atom increases considerably in the carburising flame in spite of the simultaneous increase in the background radiation. This may be due to displacement of the equilibrium $MgO \rightleftharpoons Mg$ in the flame owing to its stronger reducing effect, the concentration of magnesium atoms therefore being increased. This explanation is supported by the results of tests performed at 3721, 3811 and 3850 A, when the emission from magnesium oxide resulted in higher sensitivity with the more weakly reducing flame than with the carburising flame (see Table II).

The background radiation is extended over a wide wavelength range, but at 2852 A a sharp minimum was observed, as can be seen in Fig. 2. A slit width of about 0·03 mm, corresponding to a theoretical half-intensity band of about 1 A, was normally used with this instrument. The signal-to-background ratios were compared for slit widths of 0·03 and 0·02 mm, the latter with the use of a more sensitive photomultiplier tube (EMI 6255). However, only a slight increase in the ratio was produced with the narrower slit width.

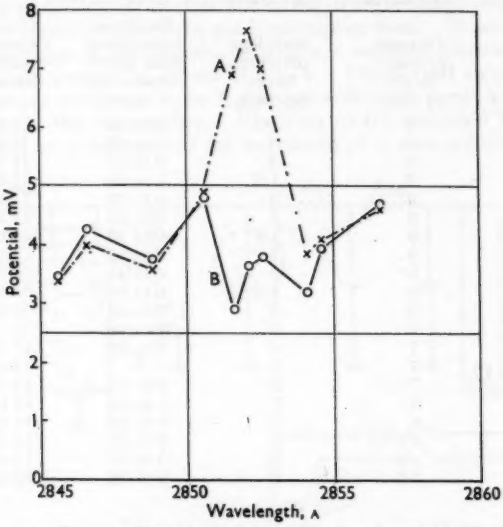


Fig. 2. Emission readings in the region of 2852 A: A, 10 p.p.m. of magnesium; B, background. Approximate readings for wavelength correction. Slit width, 0·03 mm

The flame light was masked so that only a 10-mm wide horizontal slit was left open in the wall of the burner housing. After careful trials a suitable region of the flame was chosen 11 to 25 mm above the burner tip. A suitable stable emission was produced in this position, but higher in the flame the stability was reduced.

The interfering action of certain other ions was less marked in a carburising than in a neutral flame (see also p. 250 and Table IX).

The consumption of oxygen should be reduced so that a carburising flame of suitable height can readily be produced. With each burner there are data on the pressure required to give an atomising rate of 1·5 to 2 ml per minute. It is, however, more convenient to reduce the pressure to give a consumption of about 1 ml per minute. The smaller the volume of acetylene required to produce the carburising flame, the less likelihood is there of soot being deposited on the burner tip. The flame will also be of a more suitable height and will fluctuate less.

The ejector action in some burners is greater than others. Burners having the greatest ejector action were found to be the most suitable, as the oxygen consumption could be kept lower.

A high sensitivity to magnesium was achieved with all the atomiser burners tested, provided that the following conditions were observed—

(*a*) the rate of atomisation was adjusted to about 1 ml per minute by regulating the oxygen pressure,

(*b*) the acetylene pressure was set at 5 lb per sq. inch and the gaseous mixture ignited and

(*c*) the acetylene supply was regulated by means of the fuel needle valve in the right side of the pressure-control unit, so that a yellowish white zone about 45 mm high was produced in the flame.

For the flame to give a stable emission the gas pressure must be kept constant. This is effected by maintaining a certain critical pressure ratio.[15] If the absolute gas pressure indicated on the fuel gauges of the gas cylinder and the Beckman unit are, respectively, p_1 and p_2, the ratio p_1/p_2 for acetylene should be 1·8 or over and for oxygen 1·9 or over.

In the instructions for the Beckman instruments,[16] a pressure of 0·7 atmospheres above atmospheric is recommended for the acetylene cylinder. If, for example, 5 lb per sq. inch are required for the Beckman instrument the over-pressure shown on the gauge of the acetylene cylinder should be at least 1·4 atmospheres so as to give the critical pressure ratio. Comparative values of the rate of acetylene flow at the burner tip are obtained not only from the pressures read on the fuel regulator. The flow rate is dependent also on the choke area of the needle valve in the pressure-control unit and on the design of the burner.

PREPARATION OF STANDARD AND CONTROL SOLUTIONS—

Hydrochloric acid (0·02 *M*) was the solvent in all the control tests except when aqueous solutions and acid solutions of different molarity were used for comparison. As control solutions, chlorides and acids of analytical-reagent grade were used except for the following.

Magnesium chloride—Weigh magnesium in the form of strips that have previously been scraped bright. Convert the metal to the chloride. Check the magnesium concentration by gravimetric analysis.

Calcium chloride—Weigh Iceland spar or calcium carbonate of analytical-reagent grade that has been twice precipitated as the oxalate and heated to 500° C to form the carbonate. Convert to the chloride.

STANDARD CURVES—

The magnesium emission for the 2852 A line at relatively high concentrations is weakened by the self-reversal of the atoms. This means that the gradient of the standard curve in linear co-ordinates is weakened at these concentrations. On the other hand, the signal-to-background ratio increases so that a higher precision could be achieved with more concentrated solutions.

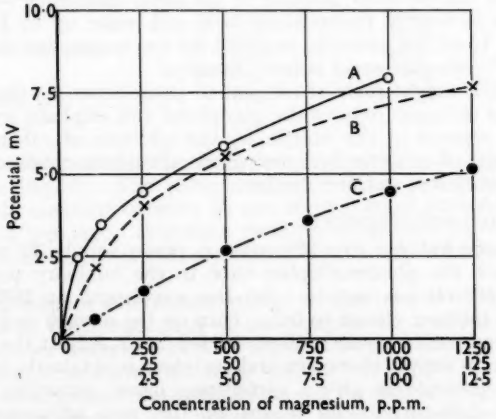


Fig. 3. Standard curves for magnesium (linear co-ordinates): curve A, 0 to 1000 p.p.m.; curve B, 0 to 125 p.p.m.; curve C, 0 to 12·5 p.p.m.

As the samples of plant material available for magnesium determination are often very small, a sensitive method was desirable, and low magnesium concentrations were consequently examined. Fig. 3 shows the standard curve in linear co-ordinates, and Fig. 4 in logarithmic co-ordinates. The curve for low magnesium concentrations is practically linear in both systems. These two curves give very similar values for the magnesium content if the points on the curve lie as close together as 1·00, 2·50, 5·00, 7·50 and 10·00 p.p.m. of magnesium.

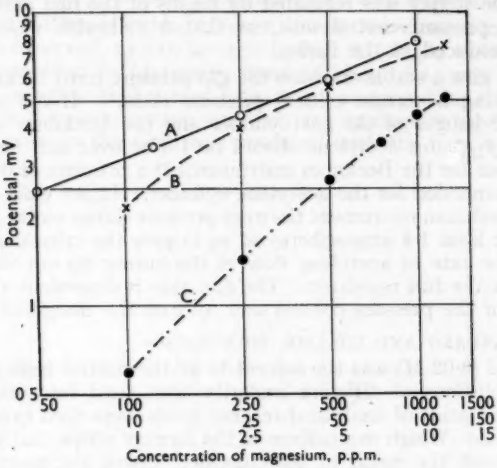


Fig. 4. Standard curves for magnesium (logarithmic co-ordinates): curve A, 50 to 1000 p.p.m.; curve B, 10 to 125 p.p.m.; curve C, 1 to 12·5 p.p.m.

PROCEDURE FOR DETERMINING MAGNESIUM—

Preliminary treatment of the plant sample—Weigh out about 1 g of the finely powdered sample and put it in a long-necked Kjeldahl flask. Digest with nitric acid and perchloric acid.[17] Remove the silica by filtration, then drive off the excess of perchloric acid by evaporation over a hot-plate and then in an oven at 200° C. In this laboratory, the sample dissolved in dilute hydrochloric acid is also repeatedly evaporated to dryness on a steam-bath to transform all the phosphate present to orthophosphate; this is desirable for certain determinations carried out on other aliquots of the same sample.[18,19] Dissolve the residue from the evaporation in 0·02 *M* hydrochloric acid and make up to 100 ml with the acid. Not more than about 15 ml are generally required for the magnesium determination. Filter the solution through Pyrex-glass-wool before atomising.

If, in a sample, the weight ratio of calcium to phosphorus (as the phosphate) is lower than 2 to 1, it is safer either to remove the phosphate and sulphate ions (see p. 251), or to increase the calcium content of the sample by the addition of calcium chloride solution. The calcium compound of analytical-reagent grade should be checked for the presence of magnesium and, if necessary, purified further.

PROCEDURE FOR TAKING MEASUREMENTS—

Connect the cathode follower to a 220-volt a.c. power supply 30 minutes before taking the readings. Connect the photomultiplier tube to the auxiliary power supply and the potentiometer to a 110-volt a.c. supply. Set the wavelength at 2852 A and the selector switch of the cathode follower circuit to 0·2. Turn on the oxygen and clean the palladium tube by introducing from below a wire of diameter 0·2 mm. Adjust the pressures to suitable values so that a constant supply of oxygen and acetylene is obtained and ignite the mixture. Adjust the acetylene pressure to give a carburising flame, according to the instructions above. Balance the dark-current by means of the zero adjustment on the cathode follower. Compensate also for the current due to the background radiation if a very low magnesium concentration is to be determined. Atomise a more concentrated solution containing, *e.g.*, 50 p.p.m. of magnesium. Make a fine adjustment of the wavelength and, if

necessary, of the entrance mirror on the monochromator, until a maximum reading is produced on the potentiometer scale. Select the slit width to give a reading of about 10 mV for about 10 p.p.m. of magnesium (a slit of about 0·03 mm is required). The difference between the reading for 10 p.p.m. of magnesium and for the solvent alone is, with a good burner, about 5 mV, which corresponds to a scale deflection of about 120 mm. The reading for the flame alone is higher than that for the solvent and for low concentrations of magnesium, presumably owing to cooling and consequent lower emission of the flame on atomisation. Make a series of readings by atomising 0·02 *M* hydrochloric acid, these serving as a check of the stability of the reading on the potentiometer scale. The readings are taken symmetrically with respect to the standard solutions, until 6 to 10 determinations of the test solution have been made. The measurement technique is described by Ehrlin-Tamm.[20]

The potentiometer needle settles slowly unless the atomising is interrupted a few times at the beginning of each new sample by raising and lowering the vessel containing the solution. When the salt solutions have been atomised, traces of deposit on the burner tip may result in a fall in the reading. This may be avoided if the palladium tube is cleaned with, for instance, *M* hydrochloric acid instead of 0·02 *M* or distilled water after each supply of salt solution.

Interfering factors

Temperature of the solution—The temperature of the solution influences the atomisation and therefore the emission. For a temperature difference of 5° C a deviation of about 3 per cent. was observed. Care should be taken, therefore, to ensure that the solutions are at room temperature before atomising and that the vessels containing the solutions are closed before and between tests.

Acid concentrations of the solutions—The tests, results of which are given in Table III, were carried out with 10·00 p.p.m. of magnesium in 0·02 *M* hydrochloric acid as a standard. It is evident from the Table that it is important to have the same concentration of acid in both the standard and unknown solutions and that 0·02 *M* concentrations of hydrochloric, nitric and perchloric acids have about the same effect on the emission.

Table III

The influence of acidity on the determination of magnesium in magnesium chloride solutions

Each solution contained 10 p.p.m. of magnesium

Substance added	Concentration of substance added, *M*	Magnesium found, p.p.m.	Error, %
Distilled water	—	10·30	+ 3·0
Hydrochloric acid ..	0·001	10·30	+ 3·0
	0·01	10·29	+ 2·9
	0·02	10·00	± 0·0
	0·10	9·77	− 2·3
Perchloric acid	0·02	9·92	− 0·8
Nitric acid	0·02	9·92	− 0·8

Various anions and cations—The presence of phosphate or sulphate ions resulted in negative errors (see Fig. 5). The phosphate error in the determination of 10 p.p.m. of magnesium was fairly constant between 50 and 200 p.p.m. of phosphorus (as the phosphate) and was about −11 per cent. Sulphate gave an error that increased continuously and was about −8 per cent. for 50 p.p.m. of sulphur (as the sulphate) and about −13 per cent. for 200 p.p.m.

Phosphorus and sulphur compounds present in plant material are changed to the phosphate and sulphate in the preparation of the samples for analysis.[17] The proportions of these ions are often high enough to involve appreciable errors in the analysis. These errors are largely eliminated if sufficient calcium is present (see Tables VI, VII, IX and XI).

The effects of some cations were studied as can be seen in Tables IV and V. The first tests showed a positive error in the presence of large quantities of pure calcium salts.

Analytical-reagent grade calcium chloride was used only for the samples that were prepared on the basis of the plant analyses, when a correction for the traces of magnesium

was also applied (see Tables IV and XI). In all other interference tests the calcium salt was freed from magnesium by precipitation twice as the oxalate.

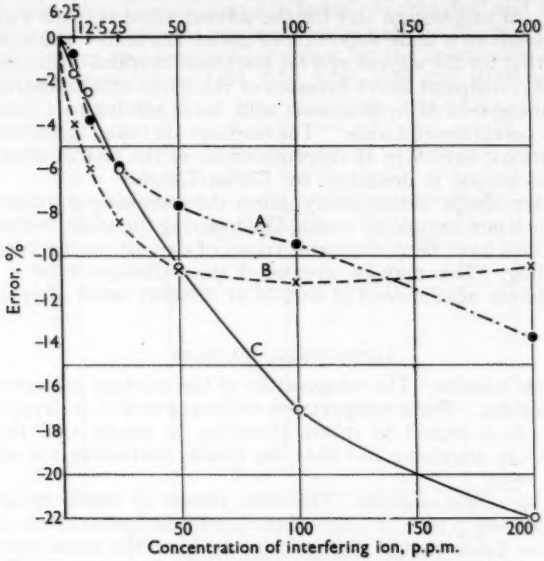


Fig. 5. Error in the determination of 10·00 p.p.m. of magnesium in the presence of interfering ions: curve A, sulphate sulphur; curve B, phosphate phosphorus; curve C, aluminium

TABLE IV

DETERMINATION OF MAGNESIUM IN THE PRESENCE OF CALCIUM CHLORIDE

All solutions were prepared with 0·02 *M* hydrochloric acid

Calcium salt taken for conversion to chloride	Calcium present, p.p.m.	Magnesium found (none added), p.p.m.	Magnesium found (10 p.p.m. added), p.p.m.	Uncorrected error, %	Corrected* error, %
AnalaR calcium carbonate ..	1000	0·29	10·60	+ 6·0	+ 3·1
	500	0·14	10·25	+ 2·5	+ 1·1
	250	0·00	10·08	+ 0·8	+ 0·8
Iceland spar	2000	0·32	—	—	—
	1000	0·10	10·29	+ 2·9	+ 1·9
	200	0·07	—	—	—
	100	—	10·04	+ 0·4	—

* Corrected with respect to magnesium content before addition of magnesium sample.

The Iceland spar was the purest substance tested. For this an error of less than 3 per cent. was obtained for 10 p.p.m. of magnesium in the presence of 1000 p.p.m. of calcium, no correction for the magnesium content before the addition of the sample being made. It is evident from this that in plant material interference due to calcium may be disregarded.

For calcium, sodium, potassium, ammonium and iron ions only small errors were observed, as can be seen in Tables IV and V. If aluminium is present in a higher concentration than a few parts per million, it should be removed before carrying out the determination (see Fig. 5).

Hutchinson[21] states that plant material contains as a rule no more than 0·002 per cent. of aluminium. At the Forest Research Institute of Sweden determinations were made of the aluminium content of spruce needles and birch leaves from an experimental area in Mölna. The spruce needles contained about 0·01 per cent. and the birch leaves about 0·001 per cent. of aluminium.

TABLE V

DETERMINATION OF MAGNESIUM IN THE PRESENCE OF VARIOUS CHLORIDES

All solutions were prepared with 0·02 *M* hydrochloric acid and contained 10 p.p.m. of added magnesium

Substance added	Concentration, p.p.m.	Magnesium found, p.p.m.	Error, %
Sodium chloride	1000 of Na	9·83	− 1·7
	100 of Na	10·18	+ 1·8
	10 of Na	9·94	− 0·6
Potassium chloride	1000 of K	9·77	− 2·3
	100 of K	9·91	− 0·9
	10 of K	9·88	− 1·2
Ammonium chloride ..	1000 of NH_3	10·30	+ 3·0
	100 of NH_3	9·75	− 2·5
	10 of NH_3	9·89	− 1·1
Aluminium chloride ..	200 of Al	7·10	− 29
	100 of Al	7·70	− 23
	10 of Al	9·65	− 3·5
	1 of Al	10·12	+ 1·2
Ferric chloride	100 of Fe	9·93	− 0·7
	10 of Fe	10·07	+ 0·7
Manganese chloride ..	250 of Mn	9·65	− 3·5
	20 of Mn	9·98	− 0·2

Manganese often occurs in plant material in concentrations similar to those of magnesium and it must be taken into account in most chemical methods of magnesium determination. In flame-spectrophotometric determination the presence of manganese does not appreciably affect the results of the analysis, this being evident from the results given in Tables V and XI.

It is clear that there was considerable negative interference from phosphate and sulphate ions and a negligible positive interference from calcium. In spite of this it was found, from the results in Table VI, that the error due to the phosphate in the presence of calcium was considerably reduced. With twice as much calcium as phosphorus (as the phosphate) the error was practically eliminated.

TABLE VI

ERROR IN DETERMINING 10 p.p.m. OF MAGNESIUM IN THE PRESENCE OF PHOSPHATE AND CALCIUM IONS

Analytical-reagent grade calcium chloride that had been further purified was used. The figures in parentheses are the number of series of results of which the mean was taken for the tabulated value.

All solutions were prepared with 0·02 *M* hydrochloric acid

Phosphorus (as phosphate), p.p.m.	Error in determining 10 p.p.m. of magnesium in the presence of— 0 p.p.m. of calcium, %	6·25 p.p.m. of calcium, %	12·5 p.p.m. of calcium, %	25 p.p.m. of calcium, %	50 p.p.m. of calcium, %	100 p.p.m. of calcium, %	100 p.p.m.* of calcium, %
0	—	—	—	—	—	+ 0·6(4)	—
6·25	—	− 1·0	− 0·3	—	—	—	—
12·5	− 4·3	—	− 3·4	− 0·6	—	—	—
25	− 6·0	—	—	− 4·5	− 1·4	—	—
50	− 9·4(8)	—	—	—	− 6·0	− 2·1(3)	− 0·7(4)

* Calcium chloride prepared from Iceland spar.

The error due to the sulphate in the presence of calcium is given in Table VII. The negative interference was almost completely removed even when the calcium content was equal to that of the sulphur (as the sulphate). In plant material calcium is often present in sufficient amounts to give these weight ratios. Tests were made with potassium and sodium instead of calcium, but no reduction of the interference by phosphate ions was effected.

TABLE VII

ERROR IN DETERMINING 10 p.p.m. OF MAGNESIUM IN THE PRESENCE OF SULPHATE AND CALCIUM IONS

Analytical-reagent grade calcium chloride that had been further purified was used. The figures in parentheses are the number of series of results of which the mean was taken for the tabulated value.

All solutions were prepared with 0·02 *M* hydrochloric acid

Sulphur (as sulphate), p.p.m.	Error in determining 10 p.p.m. of magnesium in the presence of— 0 p.p.m. of calcium, %	6·25 p.p.m. of calcium, %	12·5 p.p.m. of calcium, %	25 p.p.m. of calcium, %	50 p.p.m. of calcium, %	100 p.p.m. of calcium, %
0	—	—	—	—	—	+ 0·6(4)
6·25	− 0·8(2)	+ 4·3(2)	+ 0·7(2)	—	—	—
12·5	− 1·9(5)	—	+ 1·6(2)	+ 1·2(2)	—	—
25	− 4·7(5)	—	—	+ 0·9(2)	+ 0·4(2)	—
50	− 7·5(5)	—	—	—	− 1·7(3)	0·0(4)

Comparative determinations were carried out with 10·00 p.p.m. of magnesium and the same sample diluted to 2·00 p.p.m. It can be seen from Table VIII that for samples containing phosphate, sulphate or aluminium ions, the relative errors were considerably reduced on dilution. In the presence of iron (see Table V) the error at different dilutions was negligible.

TABLE VIII

ERROR IN DETERMINING MAGNESIUM IN THE PRESENCE OF VARIOUS IONS AT DIFFERENT DILUTIONS OF THE MAGNESIUM SOLUTION

Magnesium present, p.p.m.	Phosphorus (as phosphate) present, p.p.m.	Sulphur (as sulphate) present, p.p.m.	Calcium present, p.p.m.	Iron present, p.p.m.	Aluminium present, p.p.m.	Error in determining magnesium, %
10·00	10	—	—	—	—	− 5·0
2·00	2	—	—	—	—	+ 1·0
10·00	250	—	—	—	—	− 11
2·00	50	—	—	—	—	− 4·0
10·00	50	—	50	—	—	− 6·0
2·00	10	—	10	—	—	+ 0·5
10·00	—	—	—	480	—	− 1·3
2·00	—	—	—	96	—	+ 1·5
10·00	40	—	—	—	—	− 4·0
2·00	8	—	—	—	—	+ 1·5
10·00	50	50	50	—	—	− 8·0
2·00	10	10	10	—	—	− 3·5
10·00	—	—	—	—	200	− 27
2·00	—	—	—	—	40	− 9·5
10·00	250	—	—	—	200	− 52
2·00	50	—	—	—	40	− 29

The interference by aluminium, phosphate and sulphate ions was very dependent on the type of flame used. The effect of calcium was only slight and appears to be independent of the type of flame used (see Table IX). Interference caused by aluminium, phosphate and sulphate ions decreased as the proportion of acetylene used increased. The presence of calcium will reduce the interference of phosphate and sulphate in strongly carburising, weakly carburising and neutral flames.

REMOVAL OF THE INTERFERING SUBSTANCES—

During the course of the study a closer examination was made of whether the phosphate and sulphate ions influenced the results in the analysis of plant material (see Tables VI and VII, and Fig. 5). Attempts were therefore made to remove these ions by adsorption in an alumina column.

The adsorption columns were prepared as described by Nydahl.[22] The solvent for the samples was 0·02 *M* hydrochloric acid. Before use the column was rinsed with about 500 ml of 0·02 *M* hydrochloric acid to obtain an aluminium-free eluate. The eluate was tested for aluminium by the procedure given by Gjems and Lydersen,[23] but to the scale of 1 to 10.

TABLE IX

ERROR IN DETERMINING 10 p.p.m. OF MAGNESIUM IN THE PRESENCE OF ALUMINIUM, CALCIUM, PHOSPHATE AND SULPHATE IONS IN DIFFERENT TYPES OF FLAME

All solutions were prepared with 0·02 *M* hydrochloric acid

Oxygen pressure, lb per sq. inch	Acetylene pressure, lb per sq. inch	Type of flame	Error in determining 10 p.p.m. of magnesium in presence of— 100 p.p.m. of aluminium, %	100 p.p.m. of calcium, %	100 p.p.m. of phosphorus (as phosphate), %	100 p.p.m. of sulphur (as sulphate), %
9	5	carburising	− 12	+ 1·0	− 8	− 9
9	3·75	weak carburising	− 19	—	− 10	− 10
9	3	weak carburising	− 24	—	− 9	− 11
19	3	neutral	− 47	+ 0·5	− 21	− 27

Oxygen pressure, lb per sq. inch	Acetylene pressure, lb per sq. inch	Type of flame	Error in determining 10 p.p.m. of magnesium in presence of— 50 p.p.m. of phosphorus (as phosphate), %	50 p.p.m. of phosphorus (as phosphate) and 100 p.p.m. of calcium, %	50 p.p.m. of sulphur (as sulphate), %	50 p.p.m. of sulphur (as sulphate) and 100 p.p.m. of calcium, %
9	4·5	carburising	− 7	− 1·5	− 4	+ 0·5
9	3	weak carburising	− 9	− 3	− 8	− 1
19	3	neutral	− 19	− 6	− 16	− 2

The adsorption tests were carried out in the following manner. The alumina column was washed under pressure with a part of the solution to be analysed. Another part of the same solution was then immediately passed through the alumina column. The magnesium determinations were performed on this part directly. It was readily established, by the method described by Feigl,[24] that the phosphate ions were adsorbed. The sulphate ions presented some difficulty and could be checked only indirectly through the fall in the magnesium error.

The error obtained after treatment in the alumina column was less than −3 per cent. of the magnesium value, compared with previous figures of −7 to −15 per cent. (see Table X). Hence this method of removing the phosphate and sulphate ions seems to be efficient. Tests were also made with Dowex 2, but the adsorption of phosphate ions from a solution 0·02 *M* with respect to hydrochloric acid was negligible.

TABLE X

DETERMINATION OF 10 p.p.m. OF MAGNESIUM IN SOLUTIONS IN THE PRESENCE OF PHOSPHATE AND SULPHATE IONS BEFORE AND AFTER ADSORPTION IN ALUMINA COLUMNS

Phosphorus (as phosphate) present, p.p.m.	Sulphur (as sulphate) present, p.p.m.	Magnesium found before adsorption, p.p.m.	Error, %	Magnesium found after adsorption, p.p.m.	Error, %	Number of determinations	Standard deviation of a single determination, %
—	—	—	—	10·01	+ 0·1	3	± 2·2
50	—	8·95	− 10·5	9·87	− 1·3	3	± 1·6
100	—	8·90	− 11·0	9·74	− 2·6	2	—
—	40	9·30	− 7·0	9·96	− 0·4	13	± 0·4
—	100	9·07	− 9·3	9·80	− 2·0	11	± 1·2
—	200	8·50	− 15·0	9·71	− 2·9	6	± 1·0

THE ACCURACY AND PRECISION OF THE METHOD

Prepared samples—To check the method solutions were prepared having the same composition as those given by the analyses of plant material. The error in the magnesium determinations is given in Table XI. Correction was made for the traces of magnesium present in the calcium salt.

TABLE XI

FLAME-PHOTOMETRIC DETERMINATION OF MAGNESIUM IN SOLUTIONS PREPARED ON THE BASIS OF PLANT ANALYSES

All solutions were prepared with 0·02 *M* hydrochloric acid

Solution	Concentration	Metal added in p.p.m.— Na	K	Ca	Al	Mn	PO_4-P	SO_4-S	Mg	Magnesium found, p.p.m.	Uncorrected error, %	Corrected* error, %
I	1	10	100	100	—	50	35	—	25·00	—	—	—
I	0·8	—	—	—	—	—	—	—	20·00	20·11	+ 0·6	− 0·2
I	0·6	—	—	—	—	—	—	—	15·00	15·17	+ 1·1	+ 0·3
I	0·3	—	—	—	—	—	—	—	7·50	7·54	+ 0·5	− 0·3
II	1	10	10	100	—	25	10	—	10·00	10·45	+ 4·5	+ 2·5
III	1	5	75	100	—	—	—	—	75·00	—	—	—
III	0·2	—	—	—	—	—	—	—	15·00	14·93	− 0·5	− 0·8
III	0·1	—	—	—	—	—	—	—	7·5	7·47	− 0·4	− 0·7
IV	1	10	50	100	—	—	50	—	25·00	—	—	—
IV	0·8	—	—	—	—	—	—	—	20·00	20·14	+ 0·7	− 0·1
IV	0·3	—	—	—	—	—	—	—	7·50	7·65	+ 2·0	+ 1·2
V	1	10	150	100	2	75	40	25	30·00	—	—	—
V	0·33	—	—	—	—	—	—	—	10·00	9·80	− 2·0	− 2·7
VI	1	5	100	100	—	7·5	2	3	10·00	10·09	+ 0·9	− 1·1
VI	1	5	100	100	—	7·5	2	3	10·00	10·15	+ 1·5	− 0·5
VII	1	5	100	100	—	12·5	40	40	15·00	—	—	—
VII	0·67	—	—	—	—	—	—	—	10·00	10·08	+ 0·8	− 0·5
VIII	1	5	25	100	—	25	5	5	25·00	—	—	—
VIII	0·4	—	—	—	—	—	—	—	10·00	9·86	− 1·4	− 2·2
IX	1	10	200	100	—	125	50	10	25·00	—	—	—
IX	0·4	—	—	—	—	—	—	—	10·00	9·84	− 1·6	− 2·4
X	1	10	100	100	—	150	50	10	10·00	9·80	− 2·0	− 4·0

* Corrected for magnesium present in the added calcium.

The manganese content was comparatively high. No appreciable interference by manganese could be traced in the results of these tests. The interference tests would in that case have further reduced the magnesium content. The close agreement between the added and the found values for the magnesium could be explained only by the damping effect of the calcium on the interference by phosphate and sulphate.

Comparative chemical and flame-spectrophotometric analysis—By way of comparison, gravimetric determinations of magnesium in birch leaves were carried out. About 5 g of finely powdered leaves were weighed out for each test. The procedure used was that described on p. 246 up to the dissolution of the sample in 0·02 *M* hydrochloric acid. The magnesium was determined as the pyrophosphate by the method due principally to Kolthoff and Sandell.[25] The precipitate was heated to constant weight in a porcelain filtering crucible at 1100° C. Aluminium, iron, manganese, phosphate, calcium and magnesium were then precipitated. Ammonium salts were removed before precipitation of the magnesium.

Table XII gives the results of the comparative gravimetric and flame-spectrophotometric determinations of the magnesium in birch leaves. Manganese was present in considerable quantities. Removal of phosphate and sulphate ions had only a slight effect. The reason for this was perhaps the damping action of the calcium present on the interference. In the study of the precision a standard deviation of the single determination of ± 1·7 per cent. was found for 7 series of determinations of about 9 p.p.m. of magnesium.

Gravimetric determinations of magnesium are time-consuming. Examples of the precision and accuracy are given by Hillebrand and Lundell.[26,27] At smaller magnesium percentages the accuracy was poor.

Comparison with the precision figures of Beckman and others—The precision of the magnesium determination was studied, 10 readings being made symmetrically in relation to the standard solutions in 6 series of measurements for 10·00 p.p.m. A standard deviation of ± 0·9 per cent. was found for the mean of a series of 10 readings. The corresponding standard deviation for a solution containing 2·00 p.p.m. was ± 0·8 per cent. (10 readings in 5 series).

For between 2 and 10 p.p.m. of magnesium a standard deviation of less than 1 per cent. was found. The background reading was equivalent to about 6 p.p.m. of magnesium. The detection limit, in the sense in which the term is used by Beckman, is then 0·06 p.p.m.

Beckman[28] gives 0·2 p.p.m. at 2852 A in an oxygen - hydrogen flame. Hence the sensitivity of the method is improved, the signal-to-background ratio being 3 to 4 times greater with a corresponding reduction of the detection limit.

TABLE XII

ANALYSIS OF BIRCH LEAVES BY GRAVIMETRIC AND FLAME-PHOTOMETRIC METHODS

Organic substance and silica were removed from all samples. The values given are on a dry-weight basis

Sample number	Magnesium found by gravimetric method,* %	Mean, %	Magnesium found by flame-photometric method: before removal of phosphate and sulphate ions, %	Mean, %	Magnesium found by flame-photometric method: after removal of phosphate and sulphate ions, %	Mean, %	Calcium found, %	Manganese found, %	Phosphorus found, %	Sulphur found, %
2071	0·387, 0·372	0·380	0·371, 0·374	0·373	0·361, 0·377	0·369	1·38	0·33	0·77	—
2072	0·377, 0·370, 0·374	0·374	0·388, 0·368, 0·374	0·377	0·388, 0·387, 0·381	0·385	1·54	0·35	1·14	—
2075	0·276, 0·273	0·275	0·258 0·262, 0·264	0·261	0·267, 0·256, 0·275	0·266	1·21	0·20	0·12	0·16

* By the method of Kolthoff and Sandell.[25]

In the determination of sodium and potassium Solomon and Caton[29] obtained a precision that was slightly lower than Beckman's optimal value. They used a Beckman monochromator and the flame attachment, a multiplier phototube, an Applied Physics Corporation model 30 vibrating-reed electrometer as an amplifier and a Brown recording potentiometer.

SUMMARY

The determination of magnesium at 2852 A, when the emission was derived from the neutral magnesium atom and when a highly reducing carburising flame was used, showed a considerably higher sensitivity, whereas the interference by aluminium, phosphate and sulphate ions was lower.

In weakly and strongly reducing flames the calcium ions reduce the interference of the phosphate and sulphate ions. These ions may be removed by adsorption in an alumina column. For a concentration of 2 p.p.m. of magnesium, the interference by aluminium, phosphate and sulphate ions is less than for 10 p.p.m. of magnesium, with the same proportions of magnesium to each of the foreign ions.

The study shows that the flame-photometric determination of magnesium in plant material may be performed with satisfactory precision and accuracy in the region of 2 to 10 p.p.m.

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An Improved Formaldoxime Method for the Determination of Manganese in Plant Material

BY E. G. BRADFIELD

A method is described for the determination of manganese in plant material in which no preliminary separation of interfering elements is needed. Interference from iron and copper is eliminated by warming the solution, which results in decomposition of the formaldoxime complexes of these two metals, and precipitation of metal phosphates is prevented by using the sequestering agent N-hydroxyethylethylenediaminetriacetic acid. The method is as rapid as the normal periodate procedure and its sensitivity is about 5 times as great. The coefficient of variation is 1·2 per cent. for high concentrations of manganese and 2·3 per cent. for lower concentrations.

THE manganese content of plant material is usually determined colorimetrically by oxidation with potassium periodate to form permanganic acid.[1] Compared with most colorimetric methods the sensitivity is poor and, when used for material of low manganese content, a sample weight of 5 to 10 g is required. This weight of sample may not be available if the amount of material is limited.

Formaldoxime, which forms an orange-red colour with manganese in alkaline solutions (Mellan[2]), has been used for the determination of manganese in plant material by Sideris[3,4] and in textiles by Hamlin.[5] Both authors note interference by phosphates, which are precipitated as alkaline-earth phosphates when the solution is made alkaline, and by iron, which forms an intense violet-red colour with formaldoxime. Sideris[4] recommends the precipitation of iron as ferric phosphate and phosphates as lead phosphate in acetic acid solution, while Hamlin[5] removes iron by extracting its cupferron complex with methylene dichloride and prevents precipitation of alkaline-earth phosphates by the addition of ammonium salts and sodium pyrophosphate. Both procedures involve prior separation of iron and this increases the time required for the determination.

EXPERIMENTAL

ELIMINATION OF INTERFERENCE BY IRON—

Sideris[3] has previously shown that interference by iron can be avoided by the formation of a complex with sodium cyanide. It is, however, necessary to compensate for the greenish-

yellow colour of the cyanide complex by adding iron to the standards in amounts roughly equivalent to the content of the test solutions, a difficult procedure when the iron content of the test materials is variable. A search was therefore made for alternative agents that would selectively complex iron and prevent its reaction with formaldoxime.

Five sequestering agents (*i*) ethylenediaminetetra-acetic acid (EDTA), (*ii*) N-hydroxyethylethylenediaminetriacetic acid (HEEDTA), (*iii*) NN′-dihydroxyethylethylenediaminediacetic acid (HEEDDA), (*iv*) diethylenetriaminepenta-acetic acid (DTPA) and (*v*) 1:2-diamino*cyclo*hexanetetra-acetic acid (CDTA) were tried. Of these, only CDTA prevented iron from forming a coloured complex with formaldoxime; unfortunately, it also prevented the formation of the manganese complex. During these experiments, it was noticed that the iron - formaldoxime complex decomposed on prolonged standing, ferric hydroxide being precipitated from solutions containing EDTA and DTPA, but not from solutions containing HEEDTA and HEEDDA. It was found that the rate of decomposition was increased by warming; the reaction was therefore investigated quantitatively.

Portions of solutions of manganese and iron containing 40 μg and 300 μg, respectively, were placed in six 50-ml calibrated flasks and diluted to about 30 ml with water. To each, 5 ml of a 10 per cent. w/v solution of HEEDTA, 1 ml of diluted formaldoxime reagent (prepared from hydroxylamine sulphate and paraformaldehyde) and 2 ml of a 10 per cent. w/v solution of sodium hydroxide were added. The flasks were placed in a water bath at 65° C for 0, 10, 20, 30, 60, 120 and 240 minutes and then removed and cooled; the contents of each were diluted to the mark and the absorptions of the solutions were measured. The experiment was repeated with, in one series, 40 μg of manganese and no iron; in another 300 μg of iron and no manganese; and in a third no manganese or iron. The results are

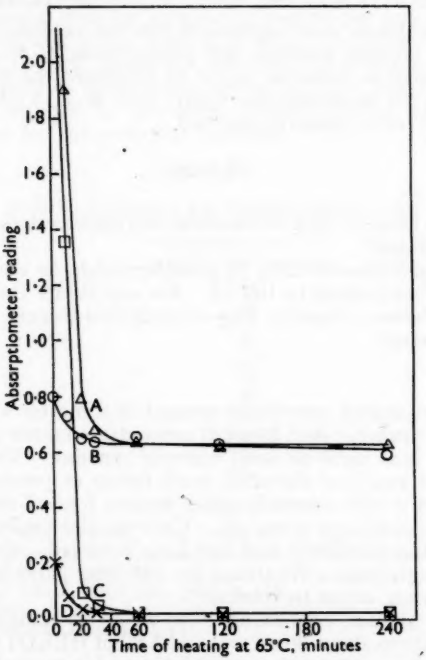

Fig. 1. Effect of heating iron and manganese with formaldoxime reaction mixture at 65° C: curve A, 40 μg of manganese + 300 μg of iron; curve B, 40 μg of manganese; curve C, 300 μg of iron; curve D, blank

shown in Fig. 1. It will be seen that, although the iron - formaldoxime complex is completely decomposed after 1 hour, the colour due to the manganese complex, after an initial

fall in intensity, is stable over the period of 1 to 3 hours. This initial fall is probably due to contamination of the reagents by iron, since the blank has a high initial absorption, which subsequently decreases on warming. The initial colour of the blank was pink, similar to that of the iron - formaldoxime complex. It is therefore possible to overcome interference by iron by warming the reaction mixture for 2 hours at 65° C before measuring the absorption.

EFFECT OF HEAT ON INTERFERING IONS—

In view of these results, the effect of warming the formaldoxime complexes of other interfering metal ions was investigated, the results being as follows—

Copper—Copper reacts with formaldoxime to give a violet coloured solution; interference can be overcome by addition of cyanide.[5] When a solution containing 100 μg of copper was warmed, the formaldoxime complex was rapidly decomposed and the solution became colourless.

Nickel—The golden brown solution produced by 100 μg of nickel with formaldoxime decomposed slowly on warming. After 2 hours at 65° C the absorption was equivalent to that of 2 μg of manganese.

Cobalt—The pale yellow-green colour of 100 μg of cobalt with formaldoxime slowly faded on warming: after 2 hours the absorption was equivalent to that of 2 μg of manganese.

Other elements—There was no colour with 100-μg amounts of zinc, cadmium, chromium, lead, molybdenum or titanium or 500 μg of aluminium, and consequently there was no interference in the determination of manganese.

EFFECT OF SEQUESTERING AGENTS ON THE PRECIPITATION OF ALKALINE-EARTH PHOSPHATES—

In plant material, calcium and magnesium are the predominant elements that form insoluble phosphates in alkaline solution and precipitation of these can be prevented by adding sufficient of a suitable chelating agent to sequester the metal ions. Experiments showed HEEDTA to be the most effective agent; 5 ml of a 10 per cent. w/v solution were adequate for the types of plant material analysed.

METHOD

REAGENTS—

HEEDTA solution—Dissolve 10 g of trisodium N-hydroxyethylethylenediaminetriacetate in water and dilute to 100 ml.

Formaldoxime reagent—Dissolve 20 g of paraformaldehyde and 55 g of hydroxylamine sulphate in boiling water and dilute to 100 ml. For use, dilute this solution 10 times.

Sodium hydroxide solution—Dissolve 10 g of analytical-reagent grade sodium hydroxide in water and dilute to 100 ml.

PROCEDURE—

Weigh 1 g of plant material, previously ground in a mortar and dried at 105° C, into a 250-ml tall Pyrex-glass beaker. Add 25 ml of concentrated nitric acid, cover with a watch-glass, and digest at low heat until no solid material remains. Then add 2·5 ml of 60 per cent. perchloric acid and continue digestion until fumes of perchloric acid are given off. If at this stage the digest is still coloured, add a further 5 ml of nitric acid and again heat until fumes of perchloric acid are given off. Uncover the beaker slightly and continue heating until nearly all the perchloric acid has been removed. Cool, add 25 ml of water, boil and filter the solution through a Whatman No. 540 filter-paper into a 100-ml Pyrex-glass calibrated flask. When cool, dilute to volume.

By pipette place an aliquot, containing 10 to 50 μg of manganese, in a 50-ml Pyrex-glass calibrated flask, dilute to about 30 ml and add 5 ml of HEEDTA solution. Add sodium hydroxide solution dropwise until any free perchloric acid is neutralised, using a wide-range pH paper as external indicator. Then add 1 ml of diluted formaldoxime reagent, followed immediately by 2 ml of sodium hydroxide solution. It is necessary to add the sodium hydroxide solution within 2 minutes of adding the reagent, since formaldoxime decomposes rapidly in dilute solutions. Remove the stopper from the flask and place it in a water bath at 65° C for 2 hours. At the end of this time remove the flask from the bath, cool the contents and dilute to 50 ml.

Measure the absorption of the solution at a wavelength of 450 mμ on a Unicam SP600 spectrophotometer, using 4-cm cells, and calculate the manganese content from a calibration graph, prepared by plotting absorption readings against the manganese contents of solutions containing 0 to 50 μg of manganese, added as a standard solution of manganese sulphate. Develop the colour of the standards in exactly the same manner as that of the samples.

Determine the blank by carrying out this procedure on the reagents only.

Results

The recovery of manganese was tested by the addition of 20 μg and 40 μg of manganese to 10-ml aliquots of a solution containing potassium, calcium, phosphate, magnesium, iron, copper, boron and zinc in such amounts that these aliquots were roughly equivalent in mineral content to an acid digest of 1·0 g of dried plant material. The results of triplicate determinations gave a mean recovery of 20·6 μg of manganese from a recovery range of 20·4 to 20·7 μg when 20 μg had been added to the aliquot. For the addition of 40 μg of manganese the mean recovery was 40·2 μg and the recovery range 39·8 to 40·6 μg.

The precision of the method was determined by carrying out a series of 10 determinations of manganese on a sample of apple leaves that had a low manganese content and a similar series on a sample of blackcurrant leaves that had a fairly high manganese content. For the apple leaves a mean content of 20·7 p.p.m. of manganese was found with a range of 20·2 to 21·6 p.p.m., the standard deviation being 0·5 and the coefficient of variation 2·3 per cent. For blackcurrant leaves the mean content was 132·1 p.p.m., with a range of 130·4 to 135·2 p.p.m., the standard deviation being 1·6 and the coefficient of variation 1·2 per cent. It will be seen that the coefficient of variation is low.

The manganese contents of a number of plant materials were then determined by the method described and compared with the values obtained by the periodate oxidation method given by Piper.[1] It will be seen from Table I, in which each result is the mean of three determinations, that there is good agreement between the two methods. For the determination of the small amounts of manganese present in apple and cauliflower leaves, only 1 g of plant material was required for the formaldoxime method whereas, for comparable accuracy, 5 g were required for the periodate method.

Table I

Manganese content of plant materials by formaldoxime and periodate methods

Material	Manganese found by formaldoxime method, p.p.m.	Manganese found by periodate method, p.p.m.
Blackcurrant leaves	129	130
Blackcurrant leaves	104	107
Apple leaves	21	20
Cauliflower leaves	19	18
Kale leaves	39	38
Wheat straw	49	51
Pasture herbage	106	109
Broccoli leaves	56	55
Strawberry leaves	325	318
Cacao leaves	564	582

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The Determination of Thorium and Lanthanons in Monazite

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The determination of thorium and lanthanons in monazite has been investigated. Oxalic acid is shown to be an unsatisfactory group precipitant because of solubility losses both of thorium and lanthanon oxalates. Precipitation with ammonium oxalate at a controlled acidity reduces these solubility losses to acceptable levels. A double precipitation with ammonium oxalate satisfactorily removes elements other than calcium from the thorium - lanthanon mixture, which can then be separated to permit thorium and lanthanons to be determined separately. Benzoic acid is a satisfactory precipitant for separating and determining thorium in such a mixture. Lanthanons can be separated from calcium and determined by precipitation as hydroxides. By using these techniques, thorium and lanthanons have been determined in a sample of South African monazite with coefficients of variation of, respectively, 1 and 0·4 per cent. The results for lanthanon content are about 1 per cent. low.

Commercially important monazites are sand concentrates from various sources and rock monazite from South Africa. Sand concentrates from Travancore and Brazil contain about 98 per cent. of "pure" monazite together with small amounts of ilmenite, quartz, etc.; other concentrates contain less monazite. The South African rock monazite is only about 70 per cent. monazite and is associated with many other minerals, particularly of the apatite group. It will therefore be appreciated that any satisfactory analysis for thorium and lanthanons must include a quantitative separation from most other elements. Other minor considerations are that the method should be rapid and suitable for routine operation by relatively unskilled personnel.

Two methods are in general use for the group separation of thorium and lanthanons from other elements. These are fluoride precipitation and oxalate precipitation, both in acid solution. The precipitation of the fluorides is not much used despite its recommendation by several authorities.[1] Factors that have contributed to its rejection are the gelatinous nature of the precipitate and the fact that the fluoride has to be converted into another form for ignition and weighing.

Oxalate precipitation has been used for monazite analysis ever since the ore achieved importance as a source of thorium, despite the fact that early investigators showed that the solubility of the oxalates in mineral acids was by no means negligible.[2] It is surprising that most methods for the determination of thorium and lanthanons in the literature ignore this fact when describing the precipitation of the oxalates.

The present investigation was originally concerned with the losses attendant on the use of oxalic acid as a group precipitant, and it was found that these were too high for the application of the method to monazite analysis. This was thought to be due to the high acidity produced by using oxalic acid and it was therefore decided to use ammonium oxalate as the precipitant so as to reduce the acidity. This reagent was found to free the thorium - lanthanon mixture from most elements except calcium. The procedure was therefore made the basis of a method for determining thorium and lanthanons in monazite and the investigation was extended to an examination of this method. In this, thorium is separated as benzoate and determined as oxide, while lanthanons are precipitated with ammonia solution and determined as oxides.

Experimental

Losses on precipitation with oxalic acid—

A solution of thorium and lanthanon ions containing the equivalent of 3·0 g of thorium oxide per litre and 45·3 g of ignited lanthanon oxides per litre was prepared from a recrystallised grade of lanthanon chloride prepared from monazite and a purified thorium nitrate. It was decided to precipitate the thorium and lanthanon oxalates from this solution under variable conditions of acidity and to introduce, in some experiments, impurities such as calcium and phosphate, which are normally present in monazite analysis.

Previous experience with oxalate precipitations showed that it is preferable to precipitate in as small a volume as possible to reduce solubility losses and from hot solution because of the nature of the precipitate. This work agrees in principle with that of Myers.[3] The following technique for precipitation was therefore adopted. Ten-millilitre aliquots of the solution (containing the equivalent of about 0·48 g of mixed oxides, an amount from 1 g of monazite) were acidified with diluted nitric acid (1 + 4) so that the acidity of the final solution ranged from 1 to 5 per cent. v/v in concentrated nitric acid. The solutions were diluted to a final volume of 100 ml. The solutions were then boiled and oxalic acid crystals were added with stirring. If calcium, phosphate or any other ions were added, the addition was made before that of the nitric acid so that any insoluble compound might be precipitated. After the addition of oxalic acid the solutions and precipitates were kept warm for 30 minutes with occasional stirring and then allowed to stand for either 2½ hours or overnight (about 18 hours), depending upon the particular experiment. The precipitated oxalates were then collected on 12·5-cm Whatman No. 542 filter-papers, allowed to drain and washed twice with 25-ml portions of oxalic acid wash solution with intermediate draining. The wash solutions were arranged to have oxalic acid and nitric acid concentrations similar to the conditions in each individual experiment, *e.g.*, a precipitate made by precipitating with 5 g of oxalic acid in 3 per cent. v/v nitric acid was washed with a solution of 5 g of oxalic acid in 100 ml of 3 per cent. v/v nitric acid.

The filtrates from these experiments were analysed for thorium and cerium in order to determine the losses. The precipitates were analysed when necessary for calcium and phosphate to determine the amount of co-precipitation. The methods used are described in the Appendix (p. 268). It is reasonable to assume that the total lanthanon loss will be about twice the cerium oxide loss.

Losses in the absence of additional ions were first investigated, the effects of variations in acidity and in the amount of oxalic acid used being examined. The results are shown in Table I. The thorium losses show that precipitation with 5 g of oxalic acid at increasing acidity causes a decrease in thorium loss, but that precipitation with 1 g of oxalic acid at increasing acidity causes an increasing thorium loss. Standing overnight causes an increase in the thorium loss. The lanthanon loss as shown by the cerium content of the filtrates shows no unexpected trends, increasing acidity causing increasing lanthanon oxalate loss. In this connection the difference between the results for 5 g and for 1 g of oxalic acid (see Table I) shows the need for the larger excess of oxalic acid. An experiment was tried with 10 g of oxalic acid and 2½-hours' standing, but the results were similar to those with 5 g of oxalic acid. The experiment was complicated by the fact that this amount of oxalic acid gave a solution close to saturation and the precipitate was contaminated with oxalic acid.

TABLE I

VARIATION OF THORIUM AND LANTHANON LOSSES WITH ACIDITY ON PRECIPITATION WITH OXALIC ACID

With 5 g of oxalic acid added—

Nitric acid present, % v/v	After standing for 2½ hours: Thorium oxide in filtrate, mg	After standing for 2½ hours: Cerium oxide in filtrate, mg	After standing overnight: Thorium oxide in filtrate, mg	After standing overnight: Cerium oxide in filtrate, mg
1	1·03	0·59	0·96	0·20
2	0·67	—	0·68	0·50
3	0·32	1·60	0·39	0·71
4	0·26	1·98	0·47	1·21
5	0·19	3·18	0·50	1·38

With 1 g of oxalic acid added—

Nitric acid present, % v/v	After standing for 2½ hours: Thorium oxide in filtrate, mg	After standing for 2½ hours: Cerium oxide in filtrate, mg	After standing overnight: Thorium oxide in filtrate, mg	After standing overnight: Cerium oxide in filtrate, mg
1	0·52	0·51	0·86	0·35
2	0·48	5·08	0·72	1·28
3	0·63	11·7	0·75	3·02
4	0·74	~17	0·88	9·67
5	0·98	~17	1·12	15·8

Oxalate precipitations were repeated in the presence of 250 mg of phosphorus pentoxide (added as a solution of potassium dihydrogen phosphate). This is approximately the amount of phosphate present in 1 g of monazite. Thorium oxide losses were determined nephelometrically[4] and are probably not reliable. The results of this series of experiments are shown in Table II. The lanthanon losses in these experiments confirmed that a large excess of oxalic acid should be used and that the acidity before precipitation should be kept to a minimum.

TABLE II

VARIATION OF THORIUM AND LANTHANON LOSSES WITH ACIDITY ON PRECIPITATION WITH OXALIC ACID IN THE PRESENCE OF ADDED PHOSPHATE

250 mg of phosphorus pentoxide (as potassium dihydrogen phosphate) present

With 5 g of oxalic acid added—

Nitric acid present, % v/v	Thorium oxide in filtrate,* mg	Cerium oxide in filtrate, mg	Phosphorus pentoxide in precipitate, mg
	After standing for 2½ hours		
1	0·13	0·47	5·4
2	0·25	0·71	5·2
3	0·25	1·54	5·0
4	0·38	1·75	5·0
5	0·38	3·13	4·2
	After standing overnight		
1	0·20	0·25	5·2
2	0·30	0·45	4·6
3	0·50	0·50	4·7
4	0·38	0·54	5·0
5	0·38	0·83	4·8

With 1 g of oxalic acid added—

Nitric acid present, % v/v	Thorium oxide in filtrate, mg	Cerium oxide in filtrate, mg	Phosphorus pentoxide in precipitate, mg
	After standing for 2½ hours		
1	—	1·64	38·3
2	—	4·62	12·7
3	—	7·08	5·1
4	—	11·4	4·7
5	—	17	3·9
	After standing overnight		
1	—	0·30	5·6
2	—	0·30	5·7
3	—	1·71	4·5
4	—	5·26	4·5
5	—	10·6	4·1

* Nephelometric determinations.

It is well known that calcium oxalate tends to be co-precipitated with lanthanon oxalates and most monazites contain some calcium. Precipitations were therefore made with the addition of various amounts of calcium oxide (added as chloride) and 250 mg of phosphate. Owing to the calcium content of the thorium and lanthanon mixture, the calcium oxide content of the solutions before precipitation was 0·9 mg in excess of the amounts added. Measurements on the oxalic acid crystals, the filter-paper and the perchloric acid indicated that these materials contributed another 0·4 mg of calcium oxide. The results of these experiments are shown in Table III. They showed that calcium, even under conditions of high acidity, is difficult to remove completely.

Separate experiments were performed in the presence of 250 mg of phosphate with 50 and 10 mg of uranium^{VI} (as uranyl nitrate), 113 mg of zirconia (as zirconyl chloride), 10 mg of lead^{II} (as nitrate), 20 mg of copper^{II} (as chloride) and 20 mg of iron^{II} (as ferrous

ammonium sulphate). These experiments showed that removal of uranium^{VI}, zirconia and iron^{II} by oxalate precipitation was excellent, but removal of lead and copper was poor. The results for lead and copper are shown in Table IV.

Table III

Variation of calcium co-precipitation with acidity on precipitation with oxalic acid in the presence of added phosphate

5 g of oxalic acid and 250 mg of phosphorus pentoxide (as potassium dihydrogen phosphate) used in each experiment

Nitric acid present, % v/v	Calcium oxide in precipitate after standing for 2½ hours, after— 5·6 mg added, mg	47·5 mg added, mg	94·1 mg added, mg	Calcium oxide in precipitate after standing overnight, after 47·5 mg added, mg
1	3·4	3·6	10·7	3·4
2	3·4	4·5	5·9	3·8
3	2·8	3·5	4·3	3·4
4	2·5	3·4	3·7	3·4
5	2·1	3·4	4·0	3·4

Table IV

Variation of lead and copper co-precipitation with acidity on precipitation with oxalic acid in the presence of added phosphate

5 g of oxalic acid and 250 mg of phosphorus pentoxide (as potassium dihydrogen phosphate) used in each experiment

Nitric acid present, % v/v	Lead in precipitate after 10 mg added, mg	Copper in precipitate after 10 mg added, mg
1	6·0	1·8
2	4·0	1·6
3	2·3	1·2
4	2·5	1·1
5	1·8	1·0

These results confirmed that copper and lead are extremely difficult elements to separate from the lanthanons by means of oxalic acid. This has been realised on a technical scale for many years.

Effect of the ammonium ion—

If the lanthanon or thorium content of a monazite, or any acid solution, has to be determined, it will have been apparent from the above work that some control of the acidity will be essential. This will mean, with monazite opened with, say, perchloric acid, that any solution before oxalate precipitation will contain considerable amounts of ammonium ion if ammonia solution is used for the adjustment of the acidity. Some precipitations were therefore carried out in the presence of ammonium chloride. The results of this work indicated significant differences from the previous work (see Table I), so a fuller investigation was made at three levels of ammonium chloride concentration, *viz.*, 2 g, 5 g and 10 g. The results are shown in Table V. Comparison of these results with those of Table I shows two important differences: (*i*) the thorium losses are extremely low; and (*ii*) the cerium losses are, in general, higher. It is obvious, too, that an increase in the ammonium chloride concentration causes an increase in the lanthanon loss.

The effect of the ammonium ion has previously been investigated for pure thorium solutions and for thorium solutions containing the equivalent of 20 mg of lanthanum oxide by Kall and Gordon.[5] Low thorium losses were experienced in their work with lanthanum present when initial pH values greater than 1·0 were employed. These pH values were attained by neutralising their standard thorium solution, which contained 5 ml of concentrated nitric acid, with ammonia solution, giving the equivalent of about 6·4 g of ammonium nitrate in each of their determinations.

TABLE V

VARIATION OF THORIUM AND LANTHANON LOSSES WITH ACIDITY ON PRECIPITATION WITH OXALIC ACID IN THE PRESENCE OF AMMONIUM CHLORIDE

5 g of oxalic acid and standing overnight used in each experiment; no phosphate added

	With 2 g of ammonium chloride present		With 5 g of ammonium chloride present		With 10 g of ammonium chloride present	
Nitric acid present, % v/v	Thorium oxide in filtrate, mg	Cerium oxide in filtrate, mg	Thorium oxide in filtrate, mg	Cerium oxide in filtrate, mg	Thorium oxide in filtrate, mg	Cerium oxide in filtrate, mg
1	nil	0·30	0·02	0·44	0·02	0·84
2	0·02	0·42	0·01	0·45	0·03	1·24
3	nil	0·64	0·01	0·45	0·02	1·78
4	0·01	0·87	0·04	0·81	0·03	2·82
5	0·02	1·11	0·02	1·91	0·03	3·53

Although the lanthanon loss is higher in the presence of ammonium ion, it appeared that it could be kept to a satisfactorily low level so that, in view of the low thorium loss, it was decided to investigate the use of ammonium oxalate as a precipitant. Preliminary work indicated that, although the thorium loss was quite considerable at 1 and 2 per cent. v/v nitric acid concentrations, the recovery of lanthanons was excellent, as shown by the results in Table VI.

TABLE VI

VARIATION OF THORIUM AND LANTHANON LOSSES WITH ACIDITY ON PRECIPITATION WITH AMMONIUM OXALATE

5 g of ammonium oxalate used in the presence of 47·5 mg of calcium oxide (as chloride) and 250 mg of phosphorus pentoxide (as potassium dihydrogen phosphate)

	After standing for 2½ hours			
Nitric acid present, % v/v	Thorium oxide in filtrate, mg	Cerium oxide in filtrate, mg	Calcium oxide in precipitate, mg	Phosphorus pentoxide in precipitate, mg
1	15·0	1·51	35	14·5
2	4·33	0·52	45	2·0
3	0·18	0·34	37	0·6
4	0·17	0·34	27	0·5
5	0·25	0·50	5·7	0·6
	After standing overnight			
1	13·7	1·01	45	2·6
2	3·1	0·45	43	2·4
3	0·26	0·37	42	1·0
4	0·06	0·50	32	1·2
5	0·10	0·76	5·1	1·5

The precipitates obtained in these experiments were washed with similar solutions to those used in the oxalic acid precipitations except that ammonium oxalate was used instead of oxalic acid. It was found that, with concentrations of ammonium oxalate above 3 per cent. w/v in 4 and 5 per cent. nitric acid, precipitation occurred. The concentration of ammonium oxalate in the 4 and 5 per cent. nitric acid wash solutions was therefore reduced to this value.

The experiments recorded in Table VI showed that there was very little difference between a 2½-hour standing period and overnight standing. An experiment was therefore tried at 4 per cent. acidity with times of standing varying from nil to 2 hours (see Table VII). To quicken the rate of filtration Whatman No. 540 filter-papers were used instead of Whatman No. 542 filter-papers in the succeeding work; no difference was detected because of this change. It was found that a short standing time of 30 minutes was sufficient to reduce the lanthanon loss to an acceptably low level.

TABLE VII

VARIATION OF THORIUM AND LANTHANON LOSSES WITH TIME ON PRECIPITATION WITH AMMONIUM OXALATE AT 4 PER CENT. ACIDITY

5 g of ammonium oxalate added in presence of 250 mg of phosphorus pentoxide (as potassium dihydrogen phosphate)

Time, hours	Thorium oxide in filtrate, mg	Cerium oxide in filtrate, mg	Phosphorus pentoxide in precipitate, mg
0	0·30	0·97	0·7
0·5	nil	0·35	1·0
1·0	0·04	0·30	0·9
1·5	0·02	0·24	0·6
2·0	0·05	0·18	1·0

This work indicated ammonium oxalate precipitation of thorium and lanthanons to be a more satisfactory method than oxalic acid precipitation, because both thorium and lanthanon losses are kept very low. It was therefore decided to adopt ammonium oxalate precipitation as a group separation for thorium and lanthanons in the analysis of monazite, and the investigation was extended to cover a scheme for the analysis of monazite based on this step. Having obtained thorium and lanthanons as a group, it is then necessary to separate and determine thorium and lanthanons separately.

ANALYSIS OF MONAZITE—

Determination of thorium—Many reagents have been proposed for the determination of thorium. The reactions in general present a similar pattern: the thorium salt of the reagent is precipitated at a more acid pH than the lanthanon salts. The simplest example of this is the precipitation of thorium hydroxide by careful neutralisation, with methyl red as indicator.[6] Thorium hydroxide is precipitated at a pH of 3·6, whereas the heavy lanthanons do not commence to be precipitated until a pH of about 6 is reached. In the presence of certain organic acids the pH limits can be made more acid while still retaining the separation. This makes the separation easier in manipulation. Benzoic acid was first proposed by Kolb and Ahrle[7] and by Neish[8] for the separation of thorium from the lanthanons. Neish rejected it in favour of *m*-nitrobenzoic acid, because of the slight solubility of benzoic acid in cold water. *m*-Nitrobenzoic acid has recently been re-investigated.[9]

Benzoic acid (or ammonium benzoate; the pH of the solution is the determining factor) commences to precipitate thorium at a pH of about 1 and precipitation is complete by a pH of 2. The lanthanons do not commence to be precipitated until a pH of about 6 is reached, when they appear as a rather slimy precipitate, easily attracted to glass. At a pH of 2 benzoic acid is only slightly soluble in the cold; precipitation must therefore be made in hot, preferably boiling, solution. The only other elements precipitated at this pH are zirconium and hafnium.

The procedure adopted is essentially that of Jewsbury and Osborn,[10] who used cresol red as an internal indicator. Their procedure is preferred to that of Venkataramaniah, Rao and Rao,[11] who add boiling benzoic acid solution to the thorium solution, the pH of which has previously been adjusted with a pH meter. We have used a modification of this method in which the pH adjustment is made with cresol red before the addition of the benzoic acid. Experience in these laboratories is that some persons are unable to see the cresol red end-point in clear solution, whereas in the presence of stirred thorium benzoate a matt background is presented and the adjustment is more easily completed.

Determination of lanthanon—The ammonium oxalate group separation does not eliminate calcium completely from the thorium - lanthanon mixture (see Table VI). The filtrates from the thorium determination will therefore contain lanthanons and calcium. We have attempted to determine lanthanons in this solution by adding ammonia solution. The nature of the precipitate is poor, however, and it is very difficult, when appreciable amounts are present, to quantitatively transfer the precipitate to a filter-paper and to wash it. Five determinations of the lanthanon oxide content of our standard solution gave a mean of 0·4432 g of ignited oxide with a standard deviation of 0·0011 g. That this result is lower than the expected result of 0·4532 g of ignited oxide is probably due to losses in the double oxalate separation.

The nature of the precipitate was considerably improved by washing it back into the original beaker, dissolving it in a little nitric acid and re-precipitating with ammonia solution. Four determinations by this method gave a mean of 0·4433 g of ignited oxide with a standard deviation of 0·0005 g. This method was therefore adopted. The calcium content of these oxides was generally less than 0·0001 g. Ignition of the precipitate at 850° C for 1½ hours gave a higher result than expected from total lanthanons added. It is recommended that the ignition be carried out at a temperature of greater than 1000° C and for a time of not less than 1 hour.

While this paper was in preparation, a similar technique involving methyl oxalate precipitation and sebacate separation was put forward by Carron, Skinner and Stevens.[12] These authors found that the light lanthanon hydroxides could be quantitatively precipitated with ammonia solution in the cold. We have not been able to confirm this.

Opening the mineral—Monazite is conveniently opened by boiling with perchloric acid. The procedure adopted in these laboratories is a modification of that given by Willard and Gordon[13] and by Clinch.[14] Perchloric acid has several quite distinct advantages over sulphuric acid and alkali-fusion techniques for routine use. Sulphuric acid forms very pasty cakes with some impure monazites, *e.g.*, South African monazites. This can cause localised overheating, with the formation of thorium-containing insoluble residues.[15] The dilution with water, too, is impracticable, for the temperature must not be allowed to rise above 40° C otherwise thorium sulphate may precipitate and be lost in the insoluble residue. Silica and other materials make filtration very difficult at this stage. Neutralisation and adjustment of the acidity is also impracticable because of double-salt formation.

Fusion with alkali hydroxide or peroxide usually opens the ore completely and rapidly. But this is not necessarily an advantage, particularly if the mineral contains much tin (as in ores from Nigeria or Malaya, where tin tailings are separated for monazite) or titanium (monazites with a high ilmenite content). The alkali-fusion technique in the chromatographic procedure of Williams[16] has been found to be extremely difficult to operate in the presence of much tin or titanium.

The perchloric acid method has the advantages that superheating cannot easily occur, that silica is precipitated in a flocculent and easily filtered form and that minerals like ilmenite, cassiterite and zircon are not attacked. The technique is therefore well suited to routine work on monazite of commercial value. Thorium has been determined by the chromatographic technique on the ignited residue obtained after treating 10 g of monazite with perchloric acid. The results showed that less than 1 mg of thorium oxide was present in the total residue even when a mineral containing much tin and titanium was used. This amount corresponds to less than 0·01 per cent. of thorium oxide in the monazite, an amount within the accuracy of the method. Moreover, dilution in the presence of phosphate and acid has the advantage that zirconium will be removed as the phosphate. Any traces remaining will undoubtedly be removed by the double oxalate separation.

With the above considerations in mind, the following method for determining thorium and lanthanons in monazite was devised.

Method

Apparatus—

A 250-ml conical flask with a B29 ground-glass socket and an air condenser about 40 cm long and having an internal diameter of 1 cm with a B29 cone.

Reagents—

Perchloric acid—An approximately 70 per cent w/v solution.
Hydrochloric acid, concentrated, 11·4 N.
Hydrazine hydrochloride, crystalline.
Hydrochloric acid, diluted (1 + 3)—Mix 80 ml of concentrated hydrochloric acid with 240 ml of water; this amount is required for each determination.
Cresol red indicator—Dissolve 1 g of solid indicator in 1 litre of 60 per cent. industrial alcohol.
Concentrated ammonia solution, sp.gr. 0·880.
Nitric acid, concentrated, 16·0 N.
Ammonium oxalate, crystalline.

Ammonium oxalate wash solution—Dissolve 30 g of ammonium oxalate in 1 litre of 4 per cent. v/v nitric acid (0·48 *N*).

Benzoic acid, crystalline.

Benzoic acid wash solution—Dissolve 10 g of benzoic acid in 1 litre of boiling water and adjust with a few drops of concentrated hydrochloric acid to the just-yellow point of cresol red.

Ammonium benzoate wash solution—Treat the benzoic acid wash solution with concentrated ammonia solution until definitely ammoniacal.

PROCEDURE—

Grind the sample so that it all passes through a 120-mesh sieve. Samples believed to contain only small amounts of monazite benefit from a further grinding to — 200 mesh. Weigh 10 g and transfer to the 250-ml conical flask. Add 40 ml of perchloric acid (50 ml is preferred for samples containing much monazite) and boil under the reflux air condenser on a hot-plate for 3 hours. Boiling should be gentle at first in case the monazite froths owing to having been separated from gangue with flotation agents. Conditions should be arranged so that the perchloric acid reaches to about 10 cm below the top of the condenser. Cool carefully, remove the air condenser and add a mixture of 80 ml of water and 20 ml of concentrated hydrochloric acid. Add slowly with vigorous shaking 4 g of hydrazine hydrochloride and then heat to boiling and keep hot for 1 hour. Replace evaporated water if necessary.

Cool the flask under the tap, add a small amount of ashless floc and shake until dispersed. Filter through a 1-cm paper-pulp pad using suction. Wash the flask and the pad thoroughly with diluted hydrochloric acid (1 + 3), using 200 ml in all. Wash the pad twice with small quantities of water. Transfer the filtrate to a 500-ml calibrated flask, using the remaining 120 ml of diluted hydrochloric acid (1 + 3) and dilute to the mark with water. The filtrate at this stage should be completely clear and free from all traces of suspended matter. If it is not, filter again through a dry Whatman No. 42 filter-paper into another clean dry flask.

Transfer a 50-ml aliquot of the solution to a 400-ml beaker, add a few drops of cresol red indicator and neutralise dropwise with concentrated ammonia solution until the indicator turns to an orange hue (pH 1·8). Add 4 ml of concentrated nitric acid, stir and dilute to 100 ml. Heat to boiling and add 5 g of ammonium oxalate. Stir the solution, keep warm for 5 minutes and then allow to cool for 30 minutes in a cold-water bath.

Collect the precipitate on a 12·5-cm Whatman No. 540 filter-paper and wash with two 25-ml portions of ammonium oxalate wash solution. Transfer the precipitate and paper back to the original beaker, add 20 ml of concentrated nitric acid and 5 ml of perchloric acid and evaporate to fumes of perchloric acid. If the solution blackens near the end of the reaction add a few millilitres of nitric acid and re-fume.

Add 90 ml of water and neutralise again with ammonia solution and cresol red indicator. Add 4 ml of concentrated nitric acid, heat to boiling and precipitate with 5 g of ammonium oxalate as before. Collect, wash and destroy the precipitate and paper with perchloric and nitric acids as before.

Dilute the solution of thorium and lanthanons to 200 ml with water and add 2 g of benzoic acid. Heat to boiling. If the solution is cloudy, add concentrated hydrochloric acid dropwise until it clears. Add a few drops of cresol red indicator and neutralise with stirring with concentrated ammonia solution added dropwise until the indicator just turns yellow. If the end-point is passed, add a few drops of concentrated hydrochloric acid and repeat the neutralisation. Bring the solution to the boil again and filter rapidly through a 12·5-cm Whatman No. 541 filter-paper, collecting the filtrate in a 600-ml beaker. Reserve it for the determination of lanthanon oxide. If only traces of thorium appear to be present, keep the solution hot for 15 minutes to ensure complete precipitation. Wash the precipitate carefully with boiling benzoic acid wash solution, retaining the washes with the previous filtrate. It is essential that all filtrations should be carried out as quickly as possible and with boiling solutions. Remove the excess of moisture from the filter-paper and precipitate between sheets of filter-paper, and ignite for 1 hour or to constant weight in a platinum or porcelain crucible. Weigh as thorium oxide (ThO_2).

Heat the combined filtrate and washes from the thorium determination to boiling and add about 20 ml of concentrated ammonia solution until the solution is strongly alkaline. Collect the precipitate on a 15-cm Whatman No. 541 filter-paper washing it with ammonium benzoate wash solution. Do not remove the last traces of precipitate from the beaker with

a rubber-tipped glass rod. Allow the precipitate to drain thoroughly and wash back with a minimum of water into the original 600-ml beaker. Retain the filter-paper. Add 5 ml of concentrated nitric acid and boil so that the acid condenses on the beaker walls and dissolves any adhering lanthanon precipitate. Allow to cool somewhat, dilute to 100 ml and re-precipitate the lanthanons with concentrated ammonia solution. Collect the precipitate on the retained Whatman No. 541 filter-paper and wash it with warm water containing a few drops of ammonia solution. Drain well and ignite in a platinum or porcelain crucible at not less than 1000° C for at least 1 hour. Weigh as ignited lanthanon oxides.

Lanthanons are present in monazite in the tervalent state. By ignition of the oxides in air, cerium, praseodymium and terbium are oxidised to higher valency states, and the weighed ignited lanthanon oxide should therefore be corrected for excess of oxygen. As praseodymium and terbium are generally present in very small quantities and cerium generally forms almost half the lanthanon content of monazite, the excess of oxygen may be conveniently determined by dissolving the oxides in sulphuric acid, oxidising the solution with ammonium persulphate by the procedure of Willard and Young[17] and determining the cerium by titration.

Results and discussion

Determinations were made on the solution of thorium and lanthanons used in the experiments on oxalate separation. Four determinations gave a mean of 0·0299 g of thorium oxide with a standard deviation of 0·0002 g. Similar solutions of thorium and lanthanons were also prepared containing 47·5 mg of calcium oxide (as chloride) and 250 mg of phosphorus pentoxide (as potassium dihydrogen phosphate). These were precipitated twice with ammonium oxalate by the proposed procedure and the thorium was determined. Seven determinations gave a mean of 0·0301 g of thorium oxide with a standard deviation of 0·0002 g, a result similar to that for the determinations in which the oxalate precipitations were omitted. No determinations were made on the quantitative nature of the thorium precipitation by benzoic acid, the work of Jewsbury and Osborn[10] and Venkataramaniah *et al.*[11] being relied upon.

In view of the reported solubilities of the heavy lanthanon oxalates in ammonium oxalate solution,[18] a solution of mixed heavy lanthanon oxides in perchloric acid was treated by the procedure. Quantitative recovery was achieved.

Two solutions were prepared simulating actual monazite solutions. Solution A was prepared by boiling 100 ml of standard thorium and lanthanon solution, 0·042 g of lead nitrate, 0·160 g of copper chloride, $CuCl_2.2H_2O$, 4·1 g of 90 per cent. w/w phosphoric acid, 1·5 g of ferric chloride, $FeCl_3.6H_2O$, 1·02 g of calcium carbonate, 0·027 g of uranyl nitrate, $UO_2(NO_3)_2.6H_2O$, 2·2 g of aluminium nitrate, $Al(NO_3)_3.9H_2O$, 0·0145 g of manganese chloride, $MnCl_2.4H_2O$, and traces of nickel, chromium and magnesium chlorides, with 50 ml of 70 per cent. perchloric acid as in the method. The solution was made up to 500 ml and 50-ml aliquots taken for analysis for thorium and lanthanons. Duplicate determinations gave 0·0301 and 0·0304 g of thorium oxide and 0·4444 and 0·4461 g of ignited lanthanon oxides; 0·030 g of thorium oxide and 0·453 g of ignited lanthanon oxides were expected.

Table VIII

Repeated analyses of a standard monazite

Sample	Weight of sample, g	Thorium oxide found, %	Ignited lanthanon oxides found, %
1	10·460	6·18, 6·21	43·31, 43·42
2	11·126	6·10, 6·10	43·20, 43·26
3	9·657	6·07, 6·17	43·49, 43·29
4	10·401	6·13, 6·08	43·57, 43·67
5	10·101	6·17, 6·17	43·63, 43·49
6	9·201	6·04, 6·05	43·59, —
Mean		6·12 per cent.	43·45 per cent.
Standard deviation		0·06 per cent.	0·16 per cent.

Solution B was prepared in a similar fashion by boiling 10 ml of standard thorium and lanthanon solution, 3·7 g of ferric chloride, 1·5 g of phosphoric acid and 2·6 g of calcium carbonate with perchloric acid. This solution was diluted to 500 ml and 50-ml aliquots

were taken. Analysis gave 0·0045 and 0·0045 g of thorium oxide and 0·0449 and 0·0442 g of ignited lanthanon oxides. Expected yields were 0·0030 g of thorium oxide and 0·0453 g of lanthanon oxides; the recoveries were considered satisfactory.

In collaboration with the Radiochemical Laboratory, Chemical Research Laboratory, Teddington, a South African monazite has been prepared as a standard sample. This has been analysed six times by the given method (see Table VIII). Analysis of variance on the results of these analyses indicates a possibly significant variation of "ignited lanthanon oxides, %" between samples. This was not investigated further.

Thorium and lanthanons have also been determined in a series of monazites from different sources, with the results shown in Table IX. These results showed that the proposed method was an improvement in time and accuracy on previous techniques. Some of these were investigated by the analytical methods described in the Appendix; the results are shown in Table X.

TABLE IX

RESULTS OBTAINED BY PROPOSED PROCEDURE

Sample	Description	Thorium oxide present, %	Thorium oxide (ThO_2) found, %	Ignited lanthanon oxide found, %
1	Malayan	—	4·22	63·4
2	Nigerian	6·09*	6·08	8·4
3	Australian	6·64*	7·05	56·7
4	Nigerian	6·9*	7·09	56·4
5	South African ..	6·03*†	6·00	—
6	South African ..	6·26†	6·27	44·4
7	South African ..	6·04†	6·32	44·0
8	Indian	10·0†	10·05	59·9
9	Brazilian	6·71†	6·73	60·8

* Thorium oxide determined by Williams's method.[16]
† Thorium oxide determined by Clinch's method.[14]

TABLE X

THORIUM AND LANTHANON LOSSES IN OTHER METHODS

Solution treated contained the equivalent of 0·03 g of thorium oxide, 0·453 g of lanthanon oxides, 0·0475 g of calcium oxide and 0·25 g of phosphorus pentoxide. Acidities were varied and additional reagents were added according to the actual methods

Method	Thorium oxide in filtrate, mg	Cerium oxide in filtrate, mg	Calcium oxide in precipitate, mg	Phosphorus pentoxide in precipitate, mg
Schoeller and Powell[19]—				
1st precipitation	0·90	1·34, 0·84	7·6	1·0
2nd precipitation	0·46	1·76	3·1	n.d.
Willard and Gordon[13]—				
(one precipitation only examined) ..	0·88, 0·64	n.d.	1·8, 1·8	0·9, 0·9
Carron, Skinner and Stevens[12]—				
1st precipitation*	0·13, 0·14	1·77, 1·62	n.d.	n.d.
2nd precipitation	0·08, 0·09	2·07, 1·73	43, 31	n.d.
Gordon, Vanselow and Willard[20] ..	0·77, 0·89	0·84, 0·19	1·9, 1·9	0·9, 0·9

n.d. = not determined.
* 0·025 g of phosphorus pentoxide only.

These results confirmed that the proposed method has considerably smaller thorium losses than any of the previous methods investigated. Some of the losses are so large as to introduce considerable errors into the determination of thorium by these techniques. Lanthanon losses in the proposed method are principally confined to the double oxalate separation. The presence of calcium in the ignited lanthanon oxides from some methods compensates for the loss of lanthanons.

APPENDIX

ANALYSIS OF FILTRATES—

The filtrates were transferred to 250-ml calibrated flasks and diluted to the mark; 100-ml portions were taken for cerium and thorium determinations. These were evaporated to fumes of perchloric acid with excess of nitric acid and 3 ml of 70 per cent. perchloric acid to destroy oxalic acid. The residues were transferred to 100-ml calibrated flasks and used for the determination of thorium or cerium. If the filtrates contained much phosphate or ammonium ion, the residues were diluted with 25 ml of water and heated to boiling, and the thorium and lanthanon phosphates were precipitated with ammonia solution. The precipitates were collected on Whatman No. 541 filter-papers with the aid of a little ashless floc, and washed once with water, and the papers and precipitates were transferred to their original beakers. The 10 ml of concentrated nitric acid and 3 ml of 70 per cent. perchloric acid were added to each and the solutions evaporated to fumes of perchloric acid. The fumed solutions were transferred to 100-ml calibrated flasks as above.

Determination of thorium—Thorium was determined colorimetrically as the APANS (thoronol) complex.[14] When much phosphate is present, the result will be low. It is doubtful, however, whether in any particular case the result obtained will be low by more than 25 per cent. This will mean that any high thorium loss determined in the presence of phosphate will be low, but low thorium losses will be relatively little affected.

Some determinations were made nephelometrically by the method of Grimaldi and Fairchild.[4] The determinations are not considered very satisfactory.

Determination of cerium—Cerium was determined by a modification of the method of Medalia and Byrne.[21] An addition of 3 ml of concentrated sulphuric acid was made to the calibrated flask containing the residue from the filtration evaporation together with 1 ml of 0·025 per cent. silver nitrate solution and 1·5 g of ammonium persulphate. The solution was diluted to 95 ml and the flask was heated in boiling water for 15 minutes. After being cooled and diluted to 100 ml with chloride-free water, the optical density of the solution was compared with water by using a Spekker absorptiometer, with a mercury lamp, Woods' glass filters and 1-cm cells. A standard graph was prepared in a similar fashion with cerium solutions of known concentration. Under these conditions a straight-line graph was obtained and a drum reading of 1·000 was equivalent to 6·72 mg of cerium^{IV} oxide. An addition of 250 mg of phosphorus pentoxide (as potassium dihydrogen phosphate) has no significant effect on the results.

ANALYSIS OF PRECIPITATES—

The precipitate was removed with the paper to the original beaker. After the addition of 20 ml of concentrated nitric acid and 3 ml of 70 per cent. perchloric acid, the contents of the beaker were heated to fumes of perchloric acid and diluted quantitatively to 100 ml in a calibrated flask. This solution was used for the determination of calcium and phosphate.

Determination of calcium—Calcium was determined by spraying the solution in an E.E.L. flame photometer (Evans Electroselenium Limited). Standards were prepared by using the original thorium and lanthanon solution. Hemingway[22] has shown that the flame emission of calcium in an E.E.L. flame photometer is seriously reduced in the presence of phosphate. We have found that 10 mg of phosphorus pentoxide (added as potassium dihydrogen phosphate) did not affect the standard calcium graph.

Determination of phosphate—A 2-ml aliquot of the solution was transferred to a 100-ml calibrated flask. After dilution to 75 ml, 10 ml of a 1 per cent. w/v solution of ammonium molybdate in 12·5 per cent. v/v sulphuric acid and 10 ml of a 0·2 per cent. w/v solution of hydrazine sulphate were added and mixed by shaking, and the flask was heated for 5 minutes in a bath of boiling water. After being cooled and diluted to the mark, the optical density of the solution was compared with water by using a Spekker absorptiometer, with a tungsten lamp, Ilford No. 608 filters and 1-cm cells. A calibration graph (a straight line) was prepared from a standard solution of potassium dihydrogen phosphate. Under our conditions a drum reading of 1·000 was equivalent to 0·80 mg of phosphate (as phosphorus pentoxide) in the reaction flask.

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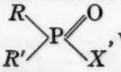
A New Approach to the Determination of Microgram Quantities of Phosphorylating or Acylating Agents

By B. Saville

A study of the reaction products from the interaction of mono*iso*nitroso-acetone with phosphorofluoridates, tetra-alkyl pyrophosphates, acetic anhydride and benzoyl chloride has revealed that the cyanide ion is formed in quantities equivalent to the amount of original agent present. By determining the cyanide by conversion to cyanogen chloride and reaction with pyridine - pyrazolone to give a blue colour, it has been possible to devise a new method for determining colorimetrically the phosphorylating or acylating agent in microgram quantities.

Recoveries of 100 $\pm$ 2 per cent. of the agents in a dilution of 10^{-4} g-equivalents per litre is possible with this procedure. Common inorganic ions do not interfere, but free halogens produce considerable interference by liberating cyanide from the mono*iso*nitrosoacetone.

There are very few known reactions that may be used as the basis of a convenient analytical procedure for the determination of phosphorylating or acylating agents, particularly if the latter are at high dilution. Although conversion of carboxylic acid esters, chlorides and anhydrides to the corresponding hydroxamic acids (identified by means of the ferric-ion complex) is a well known procedure for acylating agents,[1,2] the method lacks sensitivity. No corresponding method exists for the determination of phosphorylating agents.

Until recently no mention has been made in the literature of the general phosphorylating function of organophosphates (of the form  , where X is some electronegative group) serving as the basis of an analytical procedure, except when results have been obtained from a consideration of information about inhibition of cholinesterase. However, Epstein

and co-workers[3] and Marsh and Neale[4] have described the determination of phosphorofluoridates by using *o*-tolidine and perborate and dianisidine and peroxide, respectively. The principle of this method is that the perphosphonate derived nearly quantitatively from the organophosphate by nucleophilic attack of perhydroxyl ion will oxidise *o*-tolidine to a red-coloured dye, *i.e.*,—

$$HOOH \rightleftharpoons HOO^- + H^+$$

$$HOO^- + \underset{R\ R'}{\overset{\overset{O}{\|}}{P}}{-}F \longrightarrow HOO{-}\underset{R\ R'}{\overset{\overset{O}{\|}}{P}} + F^-$$

$$HOO{-}\underset{R\ R'}{\overset{\overset{O}{\|}}{P}} + \textit{o}\text{-tolidine} \longrightarrow \text{dye}$$

A completely different approach to the problem of determining microgram quantities of phosphorylating or acylating agents has now been examined. Green and Saville[5] have recently shown that the anions derived from acidic dissociation of many aldoximes and ketoximes are powerful nucleophilic reagents towards certain types of carbonyl and phosphoryl centres. In particular, α-oxo-aldoximes ("*iso*nitrosomethyl ketones," $R{\cdot}CO{\cdot}CH{:}NOH$) react rapidly in slightly alkaline solution with phosphorylating and acylating agents to produce an over-all hydrolysis of the agent and are themselves decomposed to hydrocyanic and carboxylic acids, the reaction proceeding stoicheiometrically as follows—

(a) *With phosphorylating agents* $\left(\begin{matrix}R'\\R''\end{matrix}\!\!>\!\!P\!\!<\!\!\begin{matrix}O\\X\end{matrix}\right)$ —

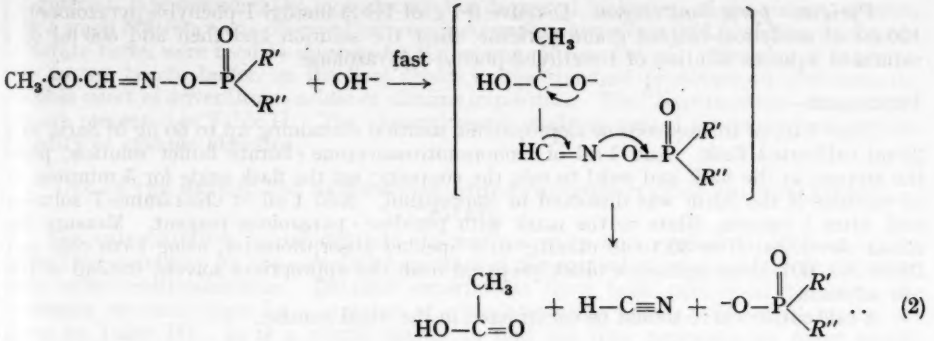
$$R{\cdot}CO{\cdot}CH{:}NOH + \begin{matrix}R'\\R''\end{matrix}\!\!>\!\!P\!\!<\!\!\begin{matrix}O\\X\end{matrix} \xrightarrow[OH^-]{H_2O} \begin{matrix}R'\\R''\end{matrix}\!\!>\!\!P\!\!<\!\!\begin{matrix}O\\OH\end{matrix} + HX + HCN + R{\cdot}COOH$$

(b) *with acylating agents* ($R{\cdot}CO{\cdot}X$) —

$$R{\cdot}CO{\cdot}CH{:}NOH + R'{\cdot}CO{\cdot}X \xrightarrow[OH^-]{H_2O} R'{\cdot}COOH + HX + HCN + R{\cdot}COOH$$

These reactions have been established by continuous titration of the acid produced to constant pH and by isolation of the products. The mechanism of the formation of cyanide in these reactions is not fully understood, although in alkaline solution the kinetics have indicated that the rate-determining step involves a bimolecular reaction between the agent and the oxime anion, in which an O-phosphorylated or O-acylated oxime is formed. This must rapidly decompose to the observed products, possibly by way of an allylic-type substitution by hydroxyl ion, *i.e.*—

$$CH_3{\cdot}CO{\cdot}CH{=}N{-}O^- + \begin{matrix}R'\\R''\end{matrix}\!\!>\!\!P\!\!<\!\!\begin{matrix}O\\X\end{matrix} \xrightarrow{\text{slow}} CH_3{\cdot}CO{\cdot}CH{=}N{-}O{-}\underset{R''}{\overset{\overset{O}{\|}}{\overset{R'}{P}}} + X^- \quad \ldots \quad (1)$$



As cyanide is formed in an amount equivalent to that of the original agent present, and the former may readily be determined by conversion to cyanogen chloride and reaction with pyridine - pyrazolone,[6] a procedure for determining phosphorylating or acylating agents is at once suggested. Bearing in mind the requirements for a rapid reaction between mono-*iso*nitrosoacetone, $CH_3{\cdot}CO{\cdot}CH:NOH$, and these agents, a general method has been developed.

General method for determining microgram quantities of phosphorylating or acylating agents

Procedure—

Equal volumes of 0·1 per cent. mono*iso*nitrosoacetone in a borate buffer solution and the solution of the agent in a suitable water-miscible solvent are mixed and allowed to react for a time sufficient for the formation of the total equivalent of cyanide. Then one volume of 0·2 per cent. chloramine T solution is added, followed by a pyridine - pyrazolone reagent to determine the cyanide. The initial red coloured solution changes to blue, and the intensity is measured after 30 to 40 minutes by means of a Spekker absorptiometer, an orange filter being used, against an appropriate blank. A calibration curve relating absorptiometer readings to concentration of agent in the test solution may be drawn.

This procedure has been applied to the determination of Sarin (*iso*propyl methyl-phosphonofluoridate) and details follow.

Method for determining Sarin in dilute aqueous or *iso*propanolic solution

Materials—

Mono*iso*nitrosoacetone was prepared by Freon's method,[7] resublimed and twice re-crystallised from a mixture of ether and light petroleum, boiling range 40° to 60° C; it had m.p. 67° C.

3-Methyl-1-phenyl-5-pyrazolone as supplied commercially was recrystallised from 95 per cent. ethanol and finally from water to give white needles, m.p. 127° C.

Bis-(3-methyl-1-phenyl-5-pyrazolone) was prepared by heating 25 g of phenylhydrazine under reflux with 17·4 g of 3-methyl-1-phenyl-3-pyrazolone in 100 ml of 95 per cent. ethanol for 4 hours. The insoluble material was filtered off and thoroughly washed with hot 95 per cent. ethanol; it had m.p. >320° C.

The phosphorus compounds were synthesised by conventional methods and acylating agents (benzoyl chloride, acetic anhydride and ethyl chloroformate) were used as supplied.

Reagents—

All solutions should be freshly prepared, although results are consistent with 1-day old solutions.

Monoisonitrosoacetone - borate buffer solution—Dissolve 0·1 g of mono*iso*nitrosoacetone in 50 ml of 0·2 M boric acid - 0·2 *M* potassium chloride solution and add 50 ml of 0·1 *M* sodium hydroxide solution.

Chloramine-T solution—Dissolve 0·2 g of chloramine T in 100 ml of water.

Pyridine - pyrazolone reagent—Dissolve 0·1 g of bis-(3-methyl-1-phenyl-5-pyrazolone) in 100 ml of analytical-reagent grade pyridine, filter the solution and then add 500 ml of a saturated aqueous solution of 1-methyl-3-phenyl-5-pyrazolone.

PROCEDURE—

Place 1 ml of the aqueous or *iso*propanolic solution containing up to 60 μg of Sarin in a 25-ml calibrated flask. Add 1 ml of mono*iso*nitrosoacetone - borate buffer solution, place the stopper in the flask and swirl to mix the contents; set the flask aside for 5 minutes, or 15 minutes if the Sarin was dissolved in *iso*propanol. Add 1 ml of chloramine-T solution and, after 1 minute, dilute to the mark with pyridine - pyrazolone reagent. Measure the colour developed after 30 to 40 minutes in a Spekker absorptiometer, using 1-cm cells and Ilford No. 607 filters against a blank prepared from the appropriate solvent instead of the test solution.

A calibration curve should be constructed in the usual manner.

CALIBRATION RESULTS—

Various amounts of Sarin in 1-ml volumes of water were used. Typical results are shown in Table I, the approximate molar concentrations in the test solutions being given for convenience.

TABLE I

CALIBRATION RESULTS

Sarin taken, μg	Approximate concentration, *M*	Absorptiometer readings	Mean
7·62	0·00005	0·135, 0·143, 0·140	0·139
15·25	0·0001	0·280, 0·282, 0·281	0·281
30·5	0·0002	0·556, 0·559, 0·553	0·556
45·7	0·0003	0·802, 0·801, 0·793	0·802
60·5	0·0004	1·010, 1·002, 1·007	1·006

RECOVERIES OF SARIN—

Known amounts of Sarin were determined by an independent worker, the calibration results given above being used. The results were as follows—

Sarin taken, μg	Sarin recovered, μg	Mean, μg
38·2	38·5, 38·4, 38·8	38·6
19·1	19·0, 19·4, 19·3	19·23

These results are reproducible to about ± 1 to 2 per cent.

EFFECT OF REACTION VARIABLES ON CYANIDE PRODUCTION

The effect of variations in the alkalinity of the mono*iso*nitrosoacetone solution, the concentration of the chloramine-T solution and the reaction times on the apparent proportion of cyanide liberated has been studied by noting the absorptiometer readings after addition of the pyridine - pyrazolone reagent. The use of phosphate buffer of pH 7·2 lowers

TABLE II

EFFECT OF REACTION VARIABLES ON APPARENT CYANIDE FORMATION FROM THE REACTION OF SARIN WITH MONO*iso*NITROSOACETONE

Solvent for mono*iso*nitrosoacetone	Sarin concentration, *M*	Absorptiometer readings after—			
		5 minutes' reaction time with		10 minutes' reaction time	
		0·2 per cent. chloramine-T solution	0·1 per cent. chloramine-T solution	0·2 per cent. chloramine-T solution	0·1 per cent. chloramine-T solution
Sodium hydroxide, 0·01*N* ..	0·0001	0·248	0·211	0·249	0·246
Sodium hydroxide, 0·01*N* ..	0·0002	0·491	0·500	0·517	0·525
Borate buffer of pH 9·10 ..	0·0002	0·560	0·483	0·553	0·473
Phosphate buffer of pH 7·2 ..	0·0002	0·146	0·129	0·269	0·272

the reaction rate considerably by reducing the degree of ionisation of the oxime, whereas only a small difference was observed between the results when either 0·01 *N* sodium hydroxide or borate buffer were used as solvents for the mono*iso*nitrosoacetone. However, it is preferable to use borate buffer, as the final choice in the standard procedure to overcome the possible effect of adventitious acidic or alkaline impurities. The "time-reaction" characteristics are presented in Table II. The absorptiometer readings quoted are proportional to the quantity of cyanide liberated.

Use of the method for determining other phosphorylating and acylating agents

The only variation introduced into the method when it is applied to different agents is the time necessary for the complete formation of the equivalent of cyanide in the reaction with mono*iso*nitrosoacetone. Detailed experiments have been performed to assess the necessary reaction times for a number of phosphorylating agents. The final results are given in Table III. It is a simple matter to find the time necessary for other similar compounds.

Table III

Reaction times for various phosphorylating and acylating agents

Agent	Solvent	Reaction time necessary for 1 ml of solution with 1 ml of 0·1 per cent. mono*iso*-nitrosoacetone in borate buffer, minutes
*iso*Propyl methylphosphonofluoridate (Sarin)	water	5
	*iso*propanol	15
Di*iso*propyl phosphorofluoridate	water	90
Tetraethyl pyrophosphate	water	25
Diethyl diethyl pyrophosphonate	water	12
Acetic anhydride	dioxan	1
Benzoyl chloride	dioxan	2
Ethyl chloroformate	dioxan	2

On substituting the reaction times given in Table III in the procedure described for determining Sarin, the calibration results for each agent have been excellent. It has also been shown that equimolar quantities of these agents give the same colour intensities to within 10 per cent. of one another and with the colour given by an equivalent amount of cyanide. Free halogens interfere, as they apparently also produce cyanide with the oxime. For this reason freshly prepared chloramine-T solutions should always be used.

Mercuric salts and rather large quantities of peroxides oxidise cyanide to cyanate and so interfere. Common inorganic ions such as chloride, nitrate, phosphate, borate and sulphate do not interfere.

Sensitivity and scope of the method

From a comparison of the working concentration ranges of the agents in the test solutions, it is claimed that the proposed method is about seven times as sensitive as the *o*-tolidine - perborate procedure for phosphorofluoridates and about thirty times as sensitive as the method for anhydrides and acid chlorides in which ferric hydroxamate is formed.

There seems little doubt that the procedure described for Sarin can be easily modified for determining other phosphorylating or acylating agents and possibly sulphonyl halides.

The compounds mentioned in this paper are used to illustrate the generality of the reaction with mono*iso*nitrosoacetone. It may be mentioned in conclusion that the procedure was developed primarily for use as a means of following the kinetic course of certain substitution reactions in which phosphorylating agents are decomposed. Preliminary results in this connection have appeared very promising. The hydrolysis rates of Sarin in borate buffers were studied and the rate constants found agreed closely with those found by using the method of Epstein *et al.*[3]

Finally, a method of determining α-oxo-aldoximes by treatment with excess of acetic anhydride, followed by the usual cyanide determination can be envisaged. This aspect has not received more than cursory attention.

Mrs. E. H. Letts rendered technical assistance in the investigations.

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Analytical Identification by Spectrophotometry

The Use of the Ionisation Constants of Weak Acids

BY A. I. BIGGS

By taking advantage of the markedly different ultra-violet absorption of phenols in acid, buffered and alkaline solutions, a method of identification is proposed for small amounts of liquids or solids, applicable even if the solubility in water is small.

THE use of ultra-violet absorption spectra for identifying organic compounds has been known for many years. In this paper it is proposed to draw attention to a variant of this technique in which instead of superimposing the complete spectrum of a suspected compound on that of a genuine sample, use is made at one wavelength only of the sensitivity of the spectra of some compounds to a change in pH.

The proposed method is based on the principles that follow. Robinson and Biggs[1] have recently used the spectrophotometer to make accurate measurements of the ionisation constants of *p*-nitrophenol. The method depends upon the fact that the ultra-violet absorption spectrum of a weak acid is often markedly dependent on pH, *i.e.*, in alkaline solution we are measuring the spectrum of the negatively charged anion of the acid, whereas if we make the pH of the solution low, say by the addition of 0·1 *N* hydrochloric acid, we are measuring the spectrum of the uncharged molecule of the weak acid. In many cases a range of wavelengths can be found in which the anion is highly absorbing, the uncharged molecule absorbing hardly at all and *vice versa* for some other range of wavelength (although it has been our experience that the larger differences between the extinction coefficients in acid and alkaline solutions are usually found at wavelengths at which it is the alkaline solution that is more highly absorbing). If a favourable wavelength of this kind is selected and measurements are made in a solution buffered at a pH close to the p*K* of the acid, then the acid will be present in the form of uncharged molecule and charged anion to about equal extents and the measured optical density will lie about half way between the optical densities observed in acid and alkaline solution. Indeed, three measurements of optical density, D, that in the buffered solution; D_1, that in acid solution (0·1 *N* hydrochloric acid); and D_2, that in alkaline solution (0·1 *N* sodium hydroxide) suffice to determine accurately α, the degree of ionisation, from the following relationship—

$$\alpha = (D-D_1)/(D_2-D_1).$$

This degree of ionisation is related to the ionisation constant by the equation—

$$K = \gamma_H m_H \frac{\gamma_A}{\gamma_{HA}} \cdot \frac{\alpha}{1-\alpha},$$

where γ denotes an activity coefficient. It is important to observe that in a given buffer solution of specified composition (and therefore of specified total ionic strength), both the activity of the hydrogen ion, $\gamma_H m_H$, and the activity coefficients of the molecule, HA, and of the anion, A, are fixed and therefore, under standard conditions, α depends on K, *i.e.*, α is a characteristic whereby an acid can be identified. As long as the concentration of the acid is low, α should be independent of this concentration and independent of the presence of impurities, provided any absorption due to the impurities does not vary with pH: α may, however, be slightly temperature dependent.

It seemed possible, therefore, that three measurements of optical density in the ultra-violet region might suffice to characterise a substance. We have already studied the absorption spectra of phenol and of nine substituted phenols[2] and we know the favourable wavelengths and pH values associated with these compounds. The proposed method of characterisation, if successful, would be valuable in that it would permit an impure specimen to be identified without prior purification and would be particularly useful for liquids or solids having low melting-points that do not form derivatives easily. Moreover, the amount of substance required is small.

We have therefore examined 35 commercial samples of these phenols, making three measurements of optical density on each, one in acid solution, one in alkaline solution and one in a suitable buffer, the temperature of each solution being 25° C. From these we calculated α, and in Table I we record these values of α together with the appropriate wavelengths and buffer solutions.

TABLE I

IDENTIFICATION OF COMMERCIAL SAMPLES OF PHENOLS

Substance	Concentration, % w/v	Buffer solution used*	Wavelength, mμ	Values of degree of ionisation for pure substances	Values of degree of ionisation found for technical samples (a)	(b)	(c)	(d)
Phenol	0·010	*A*	300	0·560	0·554	0·564	0·560	0·557
o-Cresol	0·010	*A*	300	0·400	0·403	0·396	0·401	0·405
m-Cresol	0·010	*A*	300	0·508	0·503	0·513	—	—
p-Cresol	0·010	*A*	310	0·413	0·407	0·417	0·411	0·414
o-Methoxyphenol ..	0·005	*A*	300	0·565	0·566	0·559	0·568	0·567
m-Methoxyphenol ..	0·010	*A*	300	0·738	0·736	0·740	0·738	0·733
p-Methoxyphenol ..	0·010	*A*	325	0·440	0·444	0·438	0·441	0·444
o-Nitrophenol ..	0·002	*C*	420	0·502	0·497	0·496	—	—
m-Nitrophenol ..	0·005	*B*	395	0·257	0·276	0·270	0·268	0·266
p-Nitrophenol ..	0·001	*C*	407	0·537	0·542	0·544	0·546	—

* Buffer solution *A* of pH 10·020: 0·025 *M* in sodium carbonate and 0·025 *M* in sodium hydrogen carbonate.
Buffer solution *B* of pH 7·909: 0·0083 *M* in 5:5-diethylbarbituric acid, 0·0079 *M* in the sodium salt of diethylbarbituric acid and 0·0132 *M* in sodium chloride.
Buffer solution *C* of pH 7·155: sodium dihydrogen phosphate, disodium hydrogen phosphate and sodium chloride in the molar ratios 1:1·529:1, the concentration of sodium dihydrogen phosphate being 0·005 *M*.

It will be seen that the method holds promise. If the three buffer solutions were made up as part of standard laboratory equipment, the question as to whether a commercial sample was or was not, for example, *m*-nitrophenol, could be answered in a few minutes without purification of the sample. The same is true if the sample is suspected to be *m*-methoxyphenol, with the additional advantage in this case that the same three measurements would give a positive answer should the specimen be not the *meta* compound but either the *ortho* or the *para* compound. With one compound only, such measurements would not give an unequivocal answer—*o*-cresol could not be distinguished from *p*-cresol, although it could be distinguished from phenol and from *m*-cresol.

Further work on the ultra-violet spectra of organic acids should permit Table I to be extended to a wider range of compounds, but we wish to draw attention to a method, at present applicable only to a short range of substances, that should, however, be of use to the analyst. Using previously published data,[3] we have already applied the method to barbiturates extracted from specimens of viscera.

I thank Prof. R. A. Robinson for his interest in this work.

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DEPARTMENT OF CHEMISTRY
UNIVERSITY OF MALAYA
and
DEPARTMENT OF CHEMISTRY
FEDERATION OF MALAYA

September 10th, 1956

Recommended Methods for the Analysis of Trade Effluents

PREPARED BY THE JOINT A.B.C.M. - S.A.C. COMMITTEE ON METHODS FOR THE ANALYSIS OF TRADE EFFLUENTS

Method for the Determination of Combined Nitrogen

Combined Nitrogen

COMBINED nitrogen occurs in effluents in several forms, and the methods of determination are given under the following headings, which are indicative of the mode of combination—

Ammoniacal nitrogen (free and saline ammonia).
Albuminoid nitrogen.
Organic nitrogen.
Total unoxidised nitrogen.
Nitrogen present as nitrite.
Nitrogen present as nitrate.

The terms "free" and "fixed" ammonia are frequently used in connection with ammoniacal gas liquors. "Free" ammonia is that derived on boiling the sample directly; "fixed" ammonia is that liberated on boiling, after addition of alkali, the liquid from which the "free" ammonia has first been removed. When ammoniacal liquors occur as pollutants in an effluent, this distinction ceases to be of importance, and the free and the fixed ammonia are determined together as *ammoniacal nitrogen*.

Albuminoid nitrogen is defined as the nitrogen converted to ammonia by oxidation of organic matter with an alkaline solution of potassium permanganate.

Organic nitrogen represents the total nitrogen of organic compounds, of which albuminoid nitrogen is a part.

Total unoxidised nitrogen includes nitrogen present in all forms other than nitrite and nitrate. This ought to be equivalent to the sum of the ammoniacal nitrogen plus the organic nitrogen; however, as noted in the text, the separate determination of the organic nitrogen in effluents is liable to give low results. Therefore, some stress is laid upon the importance of a direct determination of the total unoxidised nitrogen.

In general, the methods of determination consist in converting nitrogen to ammonia, removing ammonia from the sample by distillation and determining the ammonia either by nesslerisation or titration.

All results are expressed in terms of "nitrogen," as milligrams per litre of sample.

Whenever combined nitrogen in one or other of its forms is determined as ammonia, the analysis should be made in an atmosphere free from ammonia. In particular, bottles containing solutions of ammonia should not be kept in the room where the analysis is carried out.

In order to maintain uniformity in practice, several of the methods described below are substantially the same as those given in "Methods of Chemical Analysis as Applied to Sewage and Sewage Effluents," published for the Ministry of Housing and Local Government by H.M. Stationery Office.

AMMONIACAL NITROGEN

Two methods are recommended, a distillation and nesslerisation method for minute amounts of nitrogen and a distillation and titration method for larger amounts.

DISTILLATION AND NESSLERISATION METHOD

PRINCIPLE OF METHOD—

The method of determination consists in matching, visually or instrumentally, (*a*) the colour produced on adding a suitable quantity of Nessler reagent to a measured

volume of distillate with (*b*) the colour of a similarly nesslerised solution containing a known amount of standard dilute ammonium chloride solution; the two solutions must be as nearly as possible at the same temperature, preferably between 15° and 20° C.

APPARATUS—

A round-bottomed borosilicate-glass flask, having a capacity of 1 litre and fitted with an anti-splash bulb and a suitable condenser, which may be of the spiral-tube type in borosilicate glass, tin or silver.

Glass vessels, having a capacity of about 300 ml and marked at 240 ml, for collection of the distillates.

Nessler cylinders of nominal capacity 100 *ml*—As specified in British Standard 604.

Before assembling the apparatus, clean the distilling flask, anti-splash bulb and condensing tube carefully and thoroughly; afterwards, in order to free the apparatus from residual traces of ammonia, pour into the flask about 500 ml of distilled water, preferably free from ammonia, and distil until the distillate is shown to be free from ammonia by tests with Nessler reagent.

REAGENTS—

Ammonia-free distilled water—This is prepared from tap-water, but the procedure is different when the tap-water contains free chlorine.

Method (*i*)*: Preparation from tap-water containing no free chlorine*—To tap-water contained in a round-bottomed borosilicate-glass flask (having a capacity of 1 to 2 litres), add sufficient dilute sulphuric acid to make the water slightly acid to methyl orange, and then a crystal of potassium permanganate. To the neck of the flask fit an anti-splash bulb and connect the whole to a condenser. Distil with care, collecting the distillate as soon as it has been shown, by testing with Nessler reagent, to be free from ammonia. A deposit of manganese dioxide may gradually settle and should be removed between distillations in order to avoid "bumping."

Method (*ii*)*: Preparation from tap-water containing free chlorine*—To each litre of tap-water add 2 ml of a 10 per cent. solution of ferrous sulphate and sufficient sulphuric acid to give an acid reaction to methyl orange. Distil with care, using the anti-splash bulb, as described above, until one quarter of the volume remains.

Standard ammonium chloride solution A—Dissolve 3·82 g of pure dried ammonium chloride in distilled water and dilute to 1 litre, using ammonia-free distilled water.

1 ml ≡ 1 mg of ammoniacal nitrogen.

Dilute standard ammonium chloride solution B—Dilute 10 ml of solution A to 1 litre with ammonia-free distilled water. This solution should be prepared at frequent intervals.

1 ml ≡ 0·01 mg of ammoniacal nitrogen.

Nessler reagent—Dissolve 35 g of potassium iodide and 12·5 g of mercuric chloride in 300 ml of water, and add a saturated aqueous solution of mercuric chloride until a slight permanent precipitate is formed (about 44 ml will be required). Then add gradually, with frequent shaking, a solution (cooled to room temperature) of 120 g of sodium hydroxide in 150 ml of water. When the mixture has again cooled, add a further 1 ml of the saturated mercuric chloride solution and shake the mixture. Finally, dilute the mixture to 1 litre, shake it again and allow it to settle for at least 24 hours. Only the clear supernatant liquid should be used in the test. The solution is preferably stored in a rubber-stoppered bottle in the dark and only a portion of the clear supernatant liquid is transferred periodically to a small bottle for current use.

Sodium carbonate solution—A solution containing 2·5 g of sodium carbonate, Na_2CO_3, in 100 ml of distilled water.

PROCEDURE—

Size of sample—The volume of sample required in the test depends on the amount of ammonia present. For effluents containing 30 to 50 mg per litre of ammoniacal nitrogen, a suitable volume is 25 ml; for those containing substantially less, a correspondingly larger volume should be used. With effluents of high ammonia content, a preliminary dilution should be made with ammonia-free distilled water and a suitable aliquot taken for the test.

Distillation—After freeing the distillation apparatus from ammonia, empty the distilling flask and, when cool, measure into it the required volume of sample. If the sample is acid, neutralise to phenolphthalein with sodium carbonate solution; to the neutral liquid add 1 ml of sodium carbonate solution and sufficient ammonia-free distilled water to bring the volume to 500 ml. Connect the flask to the anti-splash bulb and the condenser, bring the contents quickly to the boil and distil at a rate of about 10 ml per minute, collecting 240 ml of distillate. Transfer the distillate to a 250-ml calibrated flask, dilute to the mark with ammonia-free distilled water and mix well. Continue the distillation, collecting 50-ml portions and estimating the ammoniacal nitrogen content of each by means of Nessler reagent, until the whole of the ammonia has been evolved. Usually, all the ammonia is found in the main distillate.

Determine the ammoniacal nitrogen present in the main distillate by measurement of the colour developed after the addition of Nessler reagent, as described below. The colour may be measured either visually or instrumentally.

Visual colour-comparison method—Into a series of matched 50-ml Nessler cylinders measure volumes between 0 and 5·0 ml of standard dilute ammonium chloride solution B. Into another similar Nessler cylinder measure 50 ml of the distillate (or a smaller measured volume diluted to 50 ml with ammonia-free distilled water). Fill all the cylinders to the 50-ml mark with ammonia-free distilled water, and to each add 2 ml of Nessler reagent; mix gently. Allow 10 minutes for the colour to develop and compare the colour produced by the sample with those of the standards. If the colour of the nesslerised distillate is more intense than that of the highest standard, repeat with a smaller volume of distillate. Dilution is not recommended once the Nessler reagent has been added.

Since 1 ml of standard ammonium chloride solution B is equivalent to 0·01 mg of nitrogen, then, if x ml of sample have been taken for distillation, 250 ml of the distillate have been collected, and if y ml of the distillate have been found to be equal to z ml of the standard—

$$\text{Ammoniacal nitrogen (mg per litre)} = \frac{2500\,z}{xy}.$$

To this figure should be added the figure for ammoniacal nitrogen collected in subsequent portions, if any, of the distillate.

The matching of the colour produced by Nessler reagent can also be made with standard tinted-glass discs that can be purchased. It should be noted that the disc readings are often given as ammonia, whereas the result should be recorded as nitrogen. This method has many advantages, but it is essential to prepare and use the Nessler reagent exactly in accordance with the instructions of the manufacturers of the discs. Further, each time a Nessler reagent is prepared it should be checked against standard ammonium chloride solution to ascertain whether the colours produced accord with those of the discs at the appropriate concentrations of ammonia. If not, a correction factor is necessary.

Instrumental method—The optical density of the colour may also be measured in a spectrophotometer or in an absorptiometer, using a cell of appropriate size and using a wavelength of 4670 A in a spectrophotometer or a suitable blue filter in an absorptiometer. Use water in the comparison cell. Read the number of milligrams of ammoniacal nitrogen equivalent to the observed optical density of the test solution from a previously prepared calibration graph, and so obtain the net measure of ammoniacal nitrogen in the sample.

Establish the calibration graph as follows—

Dilute appropriate measured amounts of dilute standard ammonium chloride solution B to 100 ml with ammonia-free distilled water and add to each dilution 4 ml of Nessler reagent, mix and allow to stand for 10 minutes. Measure the optical densities and construct a graph relating the optical densities to the number of milligrams of ammoniacal nitrogen.

DISTILLATION AND TITRATION METHOD

General method

PRINCIPLE OF METHOD—

In this method[1] the ammonia is distilled into a measured volume of standard sulphuric acid, the acid in excess of that required for neutralisation of the ammonia being determined by titration with standard sodium hydroxide.

APPARATUS—

The apparatus required is similar to that described under "Distillation and Nesslerisation Method," but the distilling flask need only have a capacity of 750 ml, and a double splash-head is recommended. A vertical borosilicate-glass condenser is suitable. The receiving vessel should preferably be conical and have a capacity of about 350 ml.

REAGENTS—

Sulphuric acid, N, *nitrogen-free.*

Sulphuric acid, 0·01 N—Dilute 10·0 ml of the *N* acid to 1 litre with freshly boiled and cooled ammonia-free distilled water. Store in a borosilicate-glass bottle.

Sodium hydroxide solution, N.

Sodium hydroxide solution, 0·01 N—Dilute 10·0 ml of the *N* solution to 1 litre with freshly boiled and cooled ammonia-free distilled water. Store in a borosilicate-glass aspirator fitted with a one-holed rubber stopper and provided with a suitable means for excluding atmospheric carbon dioxide, *e.g.*, a U-tube with a soda-lime guard-tube attached. This solution should be freshly prepared at frequent intervals.

Methylene blue - methyl red indicator solution—To 100 ml of a 0·1 per cent. solution of methyl red in neutral ethanol add 25 ml of a 0·1 per cent. solution of methylene blue in neutral ethanol.

Light magnesium oxide.

PROCEDURE—

After freeing the apparatus from ammonia, measure into the empty and cooled distilling flask the required volume of effluent sample (previously neutralised to phenolphthalein, if necessary), preferably containing between 0·3 and 7 mg of ammoniacal nitrogen. Dilute to 350 ml with ammonia-free distilled water, add 0·25 g of light magnesium oxide, and connect the flask to the splash-head and condenser. Place the receiving flask, containing 50·0 ml of 0·01 *N* sulphuric acid and 6 drops of methylene blue - methyl red indicator solution, below the condenser so that the end of the condenser tube reaches to the bottom of the flask. Distil at the rate of 5 to 10 ml per minute until at least 150 ml have distilled over. Boil the contents of the receiving flask and titrate hot with 0·01 *N* sodium hydroxide solution to a green end-point.

Blank determinations should be carried out occasionally and a correction made to the final titration figure for any ammonia in the reagents used.

Express the result as mg of ammoniacal nitrogen per litre of sample.

1 ml of 0·01 *N* sulphuric acid ≡ 0·14 mg of ammoniacal nitrogen.

Separate determinations of "free" and "fixed" ammonia

(*a*) *"Free" ammonia*—This may be determined by either of the foregoing methods for the determination of ammoniacal nitrogen, *omitting* the addition of sodium carbonate solution or light magnesium oxide to the effluent in the distilling flask.

(b) *"Fixed" ammonia*—After distillation of the "free" ammonia as described in (*a*) above, allow the contents of the distilling flask to cool; then add 1 ml of sodium

carbonate solution or 0·25 g of light magnesium oxide, according to the method being used, and a volume of ammonia-free distilled water equal to that removed in the distillation of the "free" ammonia. Proceed with the distillation and determination of ammoniacal nitrogen as "fixed" ammonia.

ALBUMINOID NITROGEN

THIS determination is of value in the analysis of sewage and sewage effluents, as it affords a measure of the more readily decomposable nitrogenous organic matter present. Its use in connection with trade effluents is mainly for the purpose of relating the character of the trade effluent to that of sewage.

The determination is carried out on the residue after distillation for the determination of "Ammoniacal Nitrogen" by the distillation and nesslerisation method.

REAGENTS—

In addition to the reagents listed under "Ammoniacal Nitrogen," the following is required.

Alkaline potassium permanganate solution—Dissolve 8 g of potassium permanganate in 200 ml of distilled water and separately dissolve 150 g of sodium hydroxide in 500 ml of distilled water. Mix the solutions. Dilute to about 1 litre with distilled water and boil the solution gently (preferably in an enamelled iron vessel) until the volume is reduced to about 500 ml. After cooling, dilute to 1 litre with ammonia-free distilled water.

The permanganate solution so prepared should not evolve any ammonia when tested by adding 25 ml of the solution to 250 ml of ammonia-free distilled water, distilling, and nesslerising the first 50 ml of the distillate.

PROCEDURE—

Carry out the determination on the residue after distillation for the determination of ammoniacal nitrogen (nesslerisation method).

When the residue has cooled somewhat, add 40 ml of alkaline potassium permanganate solution; then resume distillation at a rate of about 10 ml per minute and collect the distillate. Should the colour of the permanganate be destroyed on boiling, repeat the determination on a smaller quantity of sample.

Collect a number of separate fractions, *e.g.*, of 100 ml, 50 ml and 50 ml, and determine the ammonia in each by nesslerisation as described for the determination of ammoniacal nitrogen.

NOTE—Traces of ammonia in diminishing amounts are often obtained in successive fractions and experience with particular types of samples will indicate how much distillate is necessary; usually 200 ml will suffice.

ORGANIC NITROGEN

PRINCIPLE OF METHOD—

In this method,[2] any nitrite or nitrate in the effluent sample is first reduced to ammonia, which, together with any ammoniacal nitrogen already present in the sample, is removed by evaporation of the slightly alkaline solution. The organic nitrogen is then converted into ammonia by the Kjeldahl method and, after distillation, determined either by nesslerisation or titration.

Some effluents may contain organic nitrogen compounds that are not completely converted into ammonia by the Kjeldahl digestion. For example, it has been stated that nitro-, nitroso-, hydrazo-, azo- and azoxy-compounds, oximes and hydrazines are not satisfactorily converted and that cyclic nitrogen compounds, particularly those containing a pyridine ring, are only slowly broken down. It has been reported[3] that azo-, nitro- and nitroso-compounds can be satisfactorily reduced if glucose is added to the digestion mixture. Complete conversion of pyridine-ring compounds is said to require digestion for 3 to 4 hours with sulphuric acid in the presence of selenium oxychloride and metallic mercury as catalysts.[4] For compounds not amenable to either of these procedures, reduction with hydriodic acid and red phosphorus has been used.[5]

REAGENTS—

In addition to the reagents listed under "Ammoniacal Nitrogen" (nesslerisation and titration methods), the following are required.

Devarda's alloy, powdered—This must be as free from nitrogen as possible and fine enough to pass through a 200-mesh sieve.

Sodium hydroxide solution, approximately 40 per cent.—Dissolve 1500 g of pure ammonia-free sodium hydroxide in 3 litres of distilled water in an enamelled or stainless-steel vessel and boil gently for half an hour to remove any traces of ammonia.

Sulphuric acid, sp.gr. 1·84, nitrogen-free.

Phenolphthalein indicator solution, 0·1 per cent. in 50 per cent. ethanol.

PROCEDURE—

Place the required amount of effluent sample, preferably at least 100 ml (more if the content of organic nitrogen is expected to be very low), in a 300 to 350-ml round-bottomed borosilicate-glass flask having a short neck. Add 0·5 g of Devarda's alloy and 0·25 to 0·35 ml of 40 per cent. sodium hydroxide solution, and cautiously bring the whole to the boil. At the boiling-point considerable frothing is likely to occur. This should be avoided and care must be taken to prevent loss. Continue boiling until only 5 to 10 ml of liquid remain. Cool; then add 10 ml of sulphuric acid, sp. gr. 1·84, and boil the mixture fairly briskly. Continue boiling for 10 minutes after the liquid has become colourless or pale green. After cooling, add about 150 ml of ammonia-free distilled water, with continuous mixing to avoid local over-heating. Transfer the solution to a 1-litre flask, rinse the reaction flask two or three times with ammonia-free distilled water, using sufficient to dissolve any solid sulphate present, and transfer the rinsings to the larger flask. Bring the total volume to about 500 ml with ammonia-free distilled water. Add a few drops of phenolphthalein indicator solution and, taking care to keep the mixture cool to avoid loss of ammonia, add sufficient 40 per cent. sodium hydroxide solution to produce a permanent pink colour. Distil off the ammonia and determine it by one of the methods described under "Ammoniacal Nitrogen," according to the amount of nitrogen present.

Blank determinations should be carried out on the reagents and a correction made for any ammonia found.

TOTAL UNOXIDISED NITROGEN

METHOD A—IN ABSENCE OF NITRITE AND NITRATE

A measurement of the total nitrogen present in an effluent free from nitrite and nitrate is often made by separate determinations of the ammoniacal nitrogen and of the organic nitrogen and adding the amounts of the constituents found.

In certain instances, however, the sum of the results of these determinations is somewhat less than the result of a direct determination of the total nitrogen, because of the simultaneous distillation of certain undecomposed volatile nitrogenous compounds with the ammonia in the determination of ammoniacal nitrogen. Consequently, it is preferable to determine the total nitrogen present in such effluents directly by the Kjeldahl method.

REAGENTS—

In addition to the reagents listed under "Ammoniacal Nitrogen" (nesslerisation and titration methods) and "Organic Nitrogen," the following is required.

Sodium sulphate - copper sulphate mixture—Mix thoroughly, by grinding, 500 parts of anhydrous sodium sulphate and 8 parts of anhydrous copper sulphate.

PROCEDURE—

This is similar in many respects to that recommended for the determination of organic nitrogen.

Place the required amount of the effluent sample, *e.g.*, 100 ml, in a 300 to 350-ml round-bottomed borosilicate-glass flask having a short neck. Add 6 g of the sodium sulphate - copper sulphate mixture and, with care and frequent shaking, 25 ml of sulphuric acid, sp. gr. 1·84. Boil the mixture gently at first to expel water; then

continue the boiling briskly. Continue boiling the liquid for 10 minutes after the liquid has become pale green. After cooling, add about 150 ml of ammonia-free distilled water, with continuous mixing to avoid local over-heating. Transfer the solution to a 1-litre flask, rinse the reaction flask two or three times with ammonia-free distilled water, using sufficient to dissolve any solid sulphate present, and transfer the rinsings to the larger flask. Bring the total volume to about 500 ml with ammonia-free distilled water. Add a few drops of phenolphthalein indicator solution and, taking care to keep the mixture cool to avoid loss of ammonia, sufficient 40 per cent. sodium hydroxide solution to produce a permanent pink colour. Distil off the ammonia and determine it by one of the methods described under "Ammoniacal Nitrogen," according to the amount of nitrogen present, taking care to prevent excessive boiling of the solution at the commencement of the distillation.

Blank determinations should be carried out on the reagents and a correction made for any ammonia found.

Method B—In presence of nitrite and nitrate

The determination of total unoxidised nitrogen in the presence of oxidised forms cannot be readily accomplished. It is more satisfactory to reduce the oxidised forms present to ammonia, by some means such as Devarda's alloy (as described under "Organic Nitrogen"), boil off the ammonia so formed, together with the ammoniacal nitrogen originally present in the sample, and then to determine, by the Kjeldahl method described above, the organic nitrogen present in the residue.

The total unoxidised nitrogen is then taken to be the sum of the ammoniacal nitrogen and the organic nitrogen determined as described above.

NITROGEN PRESENT AS NITRITE

(Griess - Ilosvay method)

Principle of method—

The method depends upon the formation of a pink azo dye when the diazonium compound formed by the action of nitrous acid on sulphanilic acid is coupled with 1-naphthylamine.

Range—

For nitrite nitrogen contents of up to 2 mg per litre.

Reagents

Sulphanilic acid solution—Dissolve 8·0 g of sulphanilic acid in 570 ml of warm diluted acetic acid (1 + 1). When solution is complete, dilute to 1 litre with distilled water.

1-Naphthylamine solution—Dissolve 2·5 g of solid 1-naphthylamine in 290 ml of diluted acetic acid (1 + 1), warming to effect solution if necessary. When solution is complete, dilute to 500 ml with distilled water. This solution should be discarded when it becomes markedly discoloured.

Standard sodium nitrite solution A—Dissolve 0·247 g of sodium nitrite* in freshly boiled and cooled distilled water and dilute the solution to 1 litre. If kept in the dark, the solution is stable for some months.

1 ml ≡ 0·05 mg of nitrite nitrogen.

Dilute standard sodium nitrite solution B—Dilute 10·0 ml of sodium nitrite solution A to 1 litre with distilled water. This solution is not stable and must be freshly prepared.

1 ml ≡ 0·5 μg of nitrite nitrogen.

Aluminium hydroxide cream—Dissolve 125 g of aluminium potassium sulphate, $Al_2(SO_4)_3.K_2SO_4.24H_2O$, in about 1 litre of distilled water. Precipitate the aluminium hydroxide by the careful addition of ammonium hydroxide in slight excess. Allow the precipitate to settle, then wash it by decantation until the suspension is free from chloride, nitrate, nitrite and ammonia. Finally, dilute the suspension of aluminium hydroxide to 1 litre with water.

Activated charcoal, acid-washed.

* Sodium nitrite of analytical-reagent quality is only guaranteed to be 98 per cent. pure and, for extreme accuracy, it should be standardised, *e.g.*, against pure silver nitrite.

PROCEDURE—

If the effluent sample requires preliminary clarification or decolorisation, proceed as follows—

Add approximately 0·5 g of activated charcoal to 100 ml of the sample and shake the mixture; then add 1 ml of aluminium hydroxide cream, shake again and, after allowing the mixture to settle, filter it through a close-textured filter-paper, rejecting the first 20 ml of filtrate.

Into a series of matched 50-ml Nessler cylinders, measure volumes between 0 and 2·0 ml of dilute standard sodium nitrite solution B. Into another matched Nessler cylinder measure a suitable quantity of the sample, or of the filtrate resulting from the preliminary treatment described above.

Fill the cylinders to the 50-ml mark with distilled water. To each add 1 ml of sulphanilic acid solution and, after an interval of 5 minutes, 1 ml of 1-naphthylamine solution. Allow the cylinders to stand for 15 minutes in diffused light. Compare the colour produced by the sample with that of the standards.

If x ml of the sample are equivalent to y ml of dilute standard sodium nitrite solution B, then—

$$\text{Nitrite nitrogen (mg per litre)} = \frac{0{\cdot}5\,y}{x}.$$

The colour produced may also be matched with standard glass discs that can be purchased. Alternatively, the optical density may be measured in a spectrophotometer or in an absorptiometer, using a wavelength of 5461 A in the former or a suitable blue-green filter in the latter. The observed optical density is related to the number of milligrams of nitrite nitrogen by means of a previously prepared calibration graph.

When standard glass discs are used, the instructions of the manufacturers must be exactly followed. When an instrumental technique is used, rigid control of colour development and of temperature (around 20° C) is necessary.

INTERFERENCE OF CHLORIDE—

It has been stated that the presence of chloride influences the results obtained in this test. According to Klein,[6] however, chloride concentrations of less than 500 mg per litre have no appreciable effect. When it is considered that there is interference by chloride, its effect may be "drowned" by adding 1 ml of saturated sodium chloride solution to both sample and standards.

NITROGEN PRESENT AS NITRATE

EFFLUENTS containing oxidised nitrogen products usually contain both nitrite and nitrate. The former, however, is often present in only very small quantities, and in low concentration can be determined with considerable accuracy. Hence it is often practicable to arrive at a reliable figure for the nitrate concentration by determining the total oxidised nitrogen and subtracting the figure determined for nitrite.

PRINCIPLE OF METHOD—

Several methods are available for the determination of the total oxidised nitrogen. In general, these depend on reduction of the oxidised nitrogen to ammonia and subsequent determination of the ammonia contained in the solution by the methods described for the determination of ammoniacal nitrogen.

This method[7] may conveniently follow a determination of ammoniacal nitrogen involving distillation with light magnesium oxide. The oxidised compounds of nitrogen in the residue are then quantitatively reduced to ammonia on boiling with Devarda's alloy and the ammonia so produced is determined by distillation and titration or, if the content of nitrite plus nitrate is expected to be less than 4 mg of nitrogen per litre, by distillation and nesslerisation. The nitrate figure is found by deducting from the result of the determination of oxidised nitrogen the figure determined for nitrite. Evidence has been produced to show that this method gives satisfactory results irrespective of the amount of nitrite present.

REAGENTS—

In addition to the reagents listed under "Ammoniacal Nitrogen," the following is required.

Devarda's alloy, powdered—This must be as free from nitrogen as possible and fine enough to pass through a 200-mesh sieve.

PROCEDURE—

To the cooled residue in the flask after distillation of the ammoniacal nitrogen from 0·25 g of light magnesium oxide, as specified in the distillation and titration method under "Ammoniacal Nitrogen," add 1 g of powdered Devarda's alloy and sufficient ammonia-free distilled water to bring the volume in the flask to about 350 ml.

If more than 4 mg of nitrate nitrogen per litre are expected to be present, collect the ammonia in 50·0 ml of 0·01 *N* sulphuric acid and proceed as described under "Ammoniacal Nitrogen, Distillation and Titration Method."

If less than 4 mg per litre are present, proceed as described under "Ammoniacal Nitrogen, Distillation and Nesslerisation Method."

Heat the distilling flask carefully, reducing the flame when bubbles of gas become visible in order to avoid frothing and excessive spray formation as boiling begins. Boil gently for 5 to 10 minutes and then more vigorously, provided this does not cause excessive frothing. Continue the distillation until at least 150 ml of distillate have been collected. Carry out a blank determination by the method, using all the reagents.

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Notes

CALCEIN AS AN INDICATOR FOR THE TITRATION OF CALCIUM WITH ETHYLENEDIAMINETETRA-ACETATE

CALCEIN (fluorescein complexone) has been developed by Diehl and Ellingboe[1] as an indicator for calcium. It is analogous to *o*-cresolphthalein complexone[2] (called "Phthalein Complexone" by Anderegg, Flaschka, Sallmann and Schwarzenbach[3]). Calcein is more sensitive than murexide to calcium ions, but its use in titrations is similar. In neutral solutions it gives a green fluorescence, which changes on adding alkali to a pinkish orange with some residual fluorescence. In the presence of calcium, strontium, barium and magnesium ions the fluorescence is intensified and persists in alkaline solutions. The fluorescence due to magnesium is only extinguished when enough alkali is added to precipitate magnesium hydroxide completely.

The colour change on titrating calcium with ethylenediaminetetra-acetic acid (EDTA) above pH 12 is from a bright green fluorescence to a pinkish orange and in good diffuse daylight this change can be seen clearly. It is much improved, however, if thymolphthalein is added. A suitable indicator mixture consists of 0·2 g of calcein, 0·12 g of thymolphthalein and 20 g of potassium chloride ground together to a fine powder. About 10 mg of this powder are required for a titration volume of 50 ml. At this concentration the green colour is less obviously fluorescent and the residual fluorescence at the end-point does not obtrude. Under the best conditions the addition of less than 0·1 ml of 0·01 *N* EDTA solution to a volume of 50 ml gives the complete colour change from green to purple. As with murexide and Eriochrome black T, an excess of indicator must be avoided. Magnesium may also be titrated by using a calcein - thymol blue mixture if the pH is not high enough to precipitate the hydroxide, but the end-point is much inferior to that of *o*-cresolphthalein complexone or Eriochrome black T.

As none of the indicators so far suggested [murexide, calcein, 2-hydroxy-1-(2-hydroxy-4-sulpho-1-naphthylazo)-3-naphthoic acid[4]] is insensitive to magnesium, the titrations of calcium

in the presence of magnesium rely on the complete precipitation of magnesium as hydroxide. It was pointed out previously[5] that, in the presence of large proportions of magnesium, the addition of too much sodium hydroxide leads to co-precipitation of calcium on the magnesium hydroxide precipitate with consequent premature impermanent end-points. This difficulty may be avoided by using sucrose and sodium carbonate and controlling the addition of alkali.[5]

When calcein is used, a method similar to that of Bond and Tucker[5] is suitable. To a neutral aliquot of about 25 ml add 1 ml of 20 per cent. sucrose solution, 2 ml of 0·2 *M* sodium carbonate and 1 drop of 0·05 per cent. Nile blue A solution. Add 10 per cent. sodium hydroxide dropwise with mixing until the pink colour of Nile blue A is reached, and then a further 2 to 2·5 ml of alkali. Add 10 mg of calcein indicator powder and titrate with EDTA. Satisfactory titrations of calcium (99 per cent. recovery) may also be made if the concentration of calcium plus magnesium does not exceed 0·01 *N* and the alkali is added in the way just described.

The advantages of calcein over murexide are its greater sensitivity to calcium and, when thymolphthalein is added, its more satisfying colour change.

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November 1st, 1956

A RAPID METHOD FOR THE DETERMINATION OF MICROGRAM QUANTITIES OF SILVER IN LITHARGE AND RED LEAD

Litharge and red lead are used in the cupellation method for the determination of precious metals in ores. They must be as free from silver as possible, which is a likely impurity, in order that the blank value of the determination shall be low. If the lead oxide has a high silver content, it is useless for the determination of small amounts of precious metal.

A precipitation method has been described[1] in which the silver is estimated visually after the removal of all the lead by precipitation as the sulphate. We have found this method to be unsatisfactory, but, using the colorimetric reagent *p*-dimethylaminobenzylidenerhodanine, we obtained excellent results and were able to detect 0·5 μg of silver in 0·5 g of lead oxide. The procedure is very simple, but it must be emphasised that the reagent is difficult to handle successfully, as slight variations in the conditions of the test lead to inconsistent results. This has also been remarked elsewhere.[2]

Method

Reagents—

Standard silver solution—(*a*) Dilute 9·3 ml of 0·1 *N* silver nitrate solution to 1 litre with water. Then 1 ml of the solution ≡ 100 μg of silver. (*b*) Dilute 10 ml of solution (*a*) to 100 ml with water. Then 1 ml of the solution ≡ 10 μg of silver. This solution should be freshly prepared.

p-*Dimethylaminobenzylidenerhodanine solution, 0·02 per cent. in ethanol*—Prepare this by shaking the powdered reagent with absolute ethanol without heating, setting aside overnight and then filtering if necessary. Industrial methylated spirit, 74 O.P., can be used, but it is not a reliable solvent, as some batches appear to contain a denaturant that interferes with the test by destroying the background yellow colour so that a good match cannot be obtained when the colours are compared with the standards. The red colour due to the presence of silver does not appear to be so easily affected. Methanol can probably be used as a substitute for the more expensive ethanol, but, although we have used this successfully in certain of our tests, most of our investigations have been carried out with the ethanol reagent solution.

Procedure—

To 0·5 g of the oxide add 1 ml of 5 *N* nitric acid, 1 ml of water and 0·4 ml of 20-volume hydrogen peroxide and stir to dissolve. Add 5 *N* sodium hydroxide solution dropwise with stirring until the pH of the solution is just above 5·1, as indicated by a dull mauve colour on Johnson's short-range paper (pH 3·6 to 5·1). About 2 to 4 drops of the alkali should be required. Add *N* nitric acid dropwise with good stirring until the pH is slightly less than 3·6. The final drop of

N nitric acid takes the pH from above 5·1 to less than 3·6, *i.e.*, from mauve to yellow on the indicator paper. A clear solution should then be obtained. Transfer the solution with water to a 50-ml Nessler tube standing on a white tile, mix, and add 0·5 ml of a 0·02 per cent. solution of *p*-dimethylaminobenzylidenerhodanine in ethanol. Mix immediately by swirling the tube and observe it after 5 minutes. In the absence of silver a clear yellow solution is obtained. With increasing silver content the colour changes through orange to pink.

The colour obtained after 5 minutes should be compared with standards prepared by diluting aliquots of standard silver solution to 50 ml with water and adding 0·05 ml of *N* nitric acid and 0·5 ml of the reagent solution in the manner described above. The colours gradually fade on standing and the reagent and silver compound slowly flocculate out.

NOTES ON PROCEDURE—

The background colour of excess of reagent is destroyed by some oxidising agents, although the red colour of the silver compound again seems to be more permanent. The addition of 1 ml of *N* nitric acid to the standards results in an appreciable diminution of the yellow background colour. This is not entirely due to a change in the pH of the solution, because the addition of 1 ml of *N* sulphuric acid does not produce the same effect.

The reagent should be mixed with the solution immediately after it has been added. If it is allowed to remain at the top of the Nessler tube for any length of time, the strength of the yellow background colour is again decreased, possibly by reason of loss of alcohol by evaporation from the surface layer.

The mixing of the reagent solution with the test solution must be carried out by careful swirling. If vigorous stirring with a glass rod is employed, the colours are produced unevenly.[2] This may be due to flocculation of the reagent or the silver compound or both.

RESULTS

With a sample of AnalaR lead nitrate as a basis for investigating the accuracy of the method, the results for the silver content of the sample expressed as a percentage of the lead oxide content was estimated as 0·0001 per cent., *i.e.*, 0·5 μg of silver per 0·75 g of sample, where 0·75 g of sample is equivalent to 0·5 g of lead oxide. It was found that—

0·75 g of sample + 2 μg of silver matched a 2·5-μg standard.
0·75 g of sample + 4·5 μg of silver matched a 5-μg standard.
0·75 g of sample + 7 μg of silver matched a 7·5-μg standard.

We are indebted to the Directors of Hopkin and Williams Ltd. for permission to publish this Note.

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November 8th, 1956

THE DETERMINATION OF SERUM CHOLESTEROL

THE method of Zlatkis, Zak and Boyle for the determination of cholesterol in serum[1] consists in adding a sulphuric acid solution of ferric chloride to a small amount of serum dispersed in glacial acetic acid. The resulting purple colour is very stable and the reaction is more sensitive and reproducible than the Liebermann - Burchard reaction. However, there are two minor disadvantages in this otherwise very convenient procedure. The chromogenic reagent is unstable, having to be prepared freshly from stock solutions, and bilirubin increases the cholesterol values by a small amount.[2] In the proposed alternative method, proteins are precipitated by adding the serum to a stable ferric chloride - acetic acid reagent, and the colour is developed by adding sulphuric acid to a portion of the protein-free extract. The error due to bilirubin is largely eliminated, since the pigment is oxidised and mostly precipitated with the proteins. The method has the additional advantage of avoiding any possible interference arising from the presence of proteins in the final reaction mixture. This may be especially important when colour measurements are made with simple colorimeters in which wide-band filters are used, instead of with a spectrophotometer.

Method

Reagents—

Acetic acid—Purified by treating the AnalaR reagent under reflux with about 1 per cent. w/v of chromium trioxide for 2 hours and distilling, the first 10 per cent. of the distillate being discarded.

Ferric chloride - acetic acid solution—A 0·05 per cent. solution of AnalaR ferric chloride, $FeCl_3.6H_2O$, in pure acetic acid.

Sulphuric acid, M.A.R.

Stock cholesterol solution—A 1 mg per ml solution in pure acetic acid.

Cholesterol standard solution—Dilute the stock solution 1 to 25 with ferric chloride - acetic acid reagent. 5 ml of solution ≡ 0·2 mg of cholesterol.

Both cholesterol solutions are stable if kept in a cool dark place.

Procedure—

By pipette add 0·1 ml of serum or plasma to 10 ml of ferric chloride - acetic acid reagent in a glass-stoppered centrifuge tube. Mix well and set aside for 10 to 15 minutes (or over-night) for the proteins to flocculate. Spin in a centrifuge, and transfer 5 ml of clear supernatant fluid to a glass-stoppered test-tube. Into two other tubes by pipette put 5 ml of ferric chloride - acetic acid reagent (the blank) and 5 ml of cholesterol standard, respectively. To each tube add 3 ml of sulphuric acid from a burette. Place stoppers in the tubes tightly and mix the contents of each tube thoroughly by repeated inversion. Carefully loosen the stoppers and set the tubes aside for 20 to 30 minutes for full colour development. Measure the colours against the blank in an E.E.L. colorimeter (Evans Electroselenium Ltd.), using an Ilford No. 626 filter and dry colorimeter tubes, or in a spectrophotometer at 560 mμ.

Discussion

This method has been used routinely in this laboratory for over 2 years with satisfactory results. It is well within the capabilities of junior technicians. Occasional irregular results have usually been due to inadequate mixing of the rather viscous mixture of acetic and sulphuric acids, or to the use of old sulphuric acid that had absorbed excessive amounts of water. The method is very convenient for determining free and ester cholesterol after their separation on alumina, since cholesterol and its esters give equivalent colours in the Zlatkis reaction and no hydrolysis of the esters is therefore necessary. An amount of lipid extract containing about 0·3 mg of cholesterol is transferred to a column containing 0·2 g of alumina and eluted with light petroleum and benzene, as described by Kerr and Bauld,[3] except that two 10-ml fractions and two 5-ml fractions have been found to be adequate for each solvent. After evaporation to dryness, 5 ml of ferric chloride - acetic acid reagent are added to each residue and the determinations are completed as described above. Recovery of cholesterol (free plus ester) from the columns is quantitative. There is also good agreement between the total cholesterol determined directly in serum and in the corresponding lipid extract, showing that there is no loss of cholesterol on the protein precipitate.

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December 28th, 1956

A FATAL CASE OF CADMIUM POISONING

Although numerous instances of non-fatal cases of cadmium poisoning have been recorded, fatal cases fortunately appear to be very rare. Reference to the former is made in the Report of the Senior Medical Inspector of Factories and Workshops for 1933,[1] by Monier-Williams[2] and by Taylor and Hamence in a Note about lemonade ices made in cadmium-plated trays.[3] The cases reported are broadly of two types, *viz.*, those in which cadmium oxide fumes or dust are inhaled and those in which the metal is dissolved from the surface of a container. The symptoms include nausea, vomiting, gastro-enteritis and diarrhoea. Brief reference to a fatal case that occurred in 1923 is made in *The Analyst*[4] for that year, the death concerned being that of a London workman who had inhaled cadmium fumes.

The present case was different from any of its predecessors in that, although cadmium oxide was involved, the dust and fumes produced resulted from the decomposition of an organic compound of the metal.

One kilogram of cadmium propionate was being dried in two trays, one above the other, in two quantities of 500 g, in an electrically heated oven in a physics laboratory, and the initial shelf temperature was stated to have been 100° C. In the absence of personnel during the luncheon interval steps were taken to reduce the temperature to 60° C as a precautionary measure, but the cadmium propionate somehow became overheated and exploded, the fumes produced blowing open the oven door and causing the laboratory to be filled with a reddish brown smoke, which was at first mistaken for a fire. The fire brigade was accordingly summoned, several of its members subsequently suffering from sickness and headache, as initially respirators were not worn. They all received treatment with oxygen and carbon dioxide at the nearby infirmary and all but one recovered. The laboratory technician, who was the first to enter the laboratory after the explosion (in order to switch off the current heating the oven), was himself violently sick but he also recovered.

The fireman (aged 38) whose case proved fatal was taken ill the next day with wheezing and coughing and died 5½ days after exposure. After a post-mortem, specimens of urine, stomach contents, liver, lung, kidney and heart were submitted to us on behalf of the City Coroner by Dr. William Goldie, Senior Pathologist at St. James's Hospital, Leeds. Cadmium was found in each of the organs examined, the results being as follows—

Urine = 0·25 mg in 85 ml ≡ 3 mg per litre.
Stomach contents = 0·05 mg in 10 g.

For the organs—

	Weight, g	Cadmium oxide found, mg
Lungs	2350	15
Liver	2150	9
Kidneys	300	1
Heart	400	2

Method of analysis

After wet oxidation it was found possible to determine the cadmium colorimetrically as the sulphide, a minimum of 20 μg as cadmium oxide being detectable, with use of a solution of cadmium sulphate, prepared from $3CdSO_4.8H_2O$, as standard, 1 ml containing 0·01 mg of cadmium oxide. The method of Smith, Kench and Lane,[5] involving the use of aqueous ammoniacal dithizone, was also applied to the urine and confirmatory evidence was thereby obtained of the presence of cadmium.

The diphenylcarbazide spot-test also was successfully applied to the lung and liver and less successfully to the urine.

Evidence was given by one of us (C.H.M.) at the inquest, the pathological findings of Dr. Goldie being that death had resulted from asphyxia, and that the changes that had taken place were largely confined to the lungs, with fatty changes in the liver, congestion in the stomach, intestines and pancreas, and enlargement of the left ventricle of the heart.

Although the actual amounts of cadmium oxide found were small, this was not altogether surprising in view of the fact that the poison was being gradually eliminated up to death, much of it having doubtless been lost by coughing and normal excretion after the initial damage had been effected. Evidence of this was in part provided by the reddish brown colour of the urine, which after filtration gave a heavy precipitate when treated with an equal volume of 25 per cent. trichloracetic acid and exhibited heat coagulation both with and without the addition of acetic acid. (Cadmium poisoning is stated to be accompanied by proteinuria.)

It is, of course, impossible to estimate the amount of cadmium taken into the system. Nor can any reliable figure be given for the minimum lethal dose, although 1 g is quoted for this by Kaye[6] without his stating on what authority.

Although the victim in this present case may have been abnormally sensitive to cadmium oxide dust, the unfortunate results that followed upon its unintentional production only serve to emphasise the precautions to be taken against its inhalation and absorption. Standing as it does between zinc and mercury in the periodic table, due regard needs to be paid to its emetic and toxic properties.

Decomposition temperature of cadmium propionate

As it was thought by one of those concerned that the drying oven might have been set at 160° C instead of 60° C, since the thermometer scale read 10°, 20° C, etc., instead of 110°, 120° C,

etc., above the 100° C mark, an attempt was made to ascertain the decomposition temperature of cadmium propionate, which, incidentally, being the salt of a saturated fatty acid, would not be expected either to explode at 100° C or even decompose to any extent at this temperature. In fact, although decomposition readily proceeded when this white compound was heated in a test-tube over a small bunsen flame, it was found that a few crystals of it could be safely heated to 240° C in a Durham tube surrounded with liquid paraffin in a Thiele melting-point apparatus, only a slight darkening occurring at this temperature. Definite darkening was not observed until 260° C (after sulphuric acid had been submitted for liquid paraffin), the solid becoming very dark at 290° C.

Moreover, when approximately 1 g of the cadmium propionate was heated in a beaker at 100° C for ½ hour in an electric oven, in which the tray temperature was ascertained by means of a mercury-in-glass thermometer, no loss of weight took place, a loss of 0·6 per cent. in fact only occurring at 180° C. At 200° C there was a change in appearance from white to very pale yellow with a slight smell suggestive of the formation of diethyl ketone, which would be the normal result (together with cadmium oxide and carbon dioxide) of dry distillation, just as calcium acetate in similar circumstances yields acetone and calcium carbonate.

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December 7th, 1956

THE PRODUCTION OF SLIDES OF EMULSIONS

Microscope slides of emulsions are generally found difficult to make without altering the size or shape or both of the globules of the disperse phase or splitting the emulsions. For several years I have used the following method of controlling the thickness of the emulsion on the slide.

The slide is held on its edge in a wooden burette clamp, and a wire of known thickness is tied around it. A second wire is tied round at a distance from the first that is less than the diameter of the cover-slip to be used. The slide is removed from the clamp, a drop or two of the emulsion is placed between the wires and a cover-slip is lowered gently upon it. The cover-slip is then gently pressed upon the two wires, either by rolling a thick glass rod over it or by pressure from the flat surface of a soft rubber bung whose diameter is slightly less than the diameter of the cover-slip but greater than the distance between the wires.

In this way slides of any thickness can be made, and it is easy to determine the minimum thickness that will give a true picture of any emulsion.

The most useful wire gauges are as follows—

British Imperial Gauge No.	50	49	48	47	46	45	44	43	42	41	40
Diameter, inches	0·0010	0·0012	0·0016	0·0020	0·0024	0·0028	0·0032	0·0036	0·0040	0·0044	0·0048

"Haystacks"
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A. W. Middleton

January 3rd, 1957

THE DETERMINATION OF TRACES OF ZINC IN FOODSTUFFS

In the titrimetric method described in this Note the zinc in a volume of test solution containing between 0·02 and 0·10 mg is almost completely extracted by one shaking under specified conditions with a solution of dithizone in chloroform. A second extraction in which excess of the dithizone solution is used removes the small quantity of zinc remaining in the aqueous phase. A blank to which a known volume of standard zinc solution has been added (equal approximately to the quantity of zinc in the test solution), is submitted to the same extraction procedure. The mixed colours formed by the zinc - dithizone complex and the excess of dithizone of the second extractions are compared and to one or the other standard zinc solution is added from a burette, followed by shaking until the colours of the two chloroform layers match. The use of a mechanical shaker[1]

allows the two extractions to be carried out simultaneously and side by side. The method as described can be used for the determination of amounts of zinc from 20 to 200 p.p.m., but can be readily modified to determine amounts less than 20 p.p.m. An advantage lies in the use of a dithizone solution that need not be standardised.

Method

Apparatus—

Two pear-shaped 100 or 150-ml Pyrex-glass separating funnels (matched).
Burettes, of capacity 1 ml and 2 ml.
A mechanical shaker.[1]

Reagents—

Dithizone solution—Dissolve 0·025 g of dithizone per 100 ml in AnalaR chloroform. The dithizone supplied by The British Drug Houses Ltd. is suitable.

Hydrochloric acid, 5 N—Dilute 475 ml of AnalaR hydrochloric acid, sp.gr. 1·18, to approximately 1 litre. Adjust the specific gravity at 20° C to 1·081. Finally check the concentration by titration and adjust the solution to exactly 5 *N*. A check should be carried out to ensure that a mixture of 2·5 ml of the 5 *N* acid and 8 ml of 5 *N* ammonium acetate solution has a pH value of 5·0.

Sulphuric acid—The Lead-free Grade for Foodstuffs Analysis; that supplied by The British Drug Houses Ltd. is suitable.

Sodium thiosulphate solution, 10 *per cent. w/v*—Prepared from the AnalaR reagent.

Ammonium acetate solution, 5 N—Dissolve 385·4 g of AnalaR ammonium acetate in water and dilute to 1 litre.

Standard zinc solution—Dissolve 1 g of chemically pure zinc in 10 ml of concentrated hydrochloric acid (with a little water), and dilute the solution to 1 litre. Dilute 10 ml of this solution to 1 litre to give a standard solution containing 10 μg per ml.

Bromocresol green indicator solution—The solution supplied by The British Drug Houses Ltd. is suitable.

Procedure for preparing the sample solution—

Ash 5 g of the sample with 0·5 ml of concentrated sulphuric acid added, in a silica dish, first over a small flame and then to completion in a muffle furnace at a low temperature (550° C). Take up the ash in 2·5 ml of 5 *N* hydrochloric acid, add 8 ml of 5 *N* ammonium acetate solution, and transfer the solution with washings to a 100-ml calibrated flask and dilute to the mark. The pH value of this solution should be 5·0. This is the test solution.

Prepare a blank solution by evaporating completely 0·5 ml of concentrated sulphuric acid in a silica basin, then adding 2·5 ml of 5 *N* hydrochloric acid and 8 ml of 5 *N* ammonium acetate solution and diluting to 100 ml.

Procedure for an approximate estimation—

Select two pear-shaped Pyrex-glass separating funnels (of capacity 100 or 150 ml) of colourless glass having as nearly as possible the same dimensions at the "apex." Measure 1 ml of the test solution into one and 1 ml of the blank solution into the other. Add to each 1·25 ml of 5 *N* hydrochloric acid, 4 ml of 5 *N* ammonium acetate solution and 5 ml of distilled water. Mix, and add to each 1 ml of 10 per cent. w/v sodium thiosulphate solution. From a 1 or 2-ml burette add 0·5 ml of freshly prepared dithizone solution to each funnel and then 10 ml of chloroform. Shake the test solution vigorously for exactly 3 minutes. Add to the blank solution, in 0·1-ml increments, standard zinc solution, shaking vigorously for 1 minute after each addition, until the colours of the dithizone layers in the funnels match (see Note 6). Note the volume of standard zinc solution required. Then wash out the funnels.

Procedure for an accurate determination—

From the volume of zinc solution required calculate that volume of the test solution, *V*, that will contain between 20 and 100 μg of zinc. Put this volume of the test solution into one of the selected funnels, and the same volume of the blank solution into the other funnel. To each add 1 ml of 10 per cent. w/v sodium thiosulphate solution and mix well. To the blank add that volume, *Z*, of the standard zinc solution that will contain the same quantity of zinc as that estimated to be present in the volume, *V*, of the test solution. Add *Z* ml of water to the test

solution. To each funnel add the volume of dithizone solution corresponding to the zinc content as shown by the following table—

Quantity of zinc estimated to be present in V, μg	20	30	40	50	60	70	80	90
Volume of dithizone to be used for first extraction, ml	1·0	1·5	2·0	2·5	3·0	3·5	3·5	4·0

Dilute the dithizone solution to 10 ml with chloroform. Shake both separating funnels and contents for exactly 3 minutes, run off the dithizone layer, and wash out with a few drops of chloroform until the bottom layer is colourless. Now add to each funnel exactly 0·5 ml of dithizone solution diluted with 10 ml of chloroform and shake both for exactly 3 minutes. Comparison of the dithizone - chloroform layers will show one of four possibilities, the procedure for each of which is shown as follows—

Colour observed (T = test, B = blank)	Standard zinc solution (1 ml = 10 μg of Zn) to be added either to T or B so that $B = T$	Zinc content of T, ml of standard	Zinc content of sample, p.p.m.
1. $T = B$ (a match)	none to either	$T = Z$	$\frac{200\,Z}{V}$
2. B is less red than T	to B (x ml)	$T = Z + x$	$\frac{200\,(Z + x)}{V}$
3. T is less red than B	to T (y ml)	$T = Z - y$	$\frac{200\,(Z - y)}{V}$
4. T and B are violet red (both contain excess of zinc)	None to either. Run off the dithizone - chloroform layer from both and extract each with a second 0·5 ml of dithizone solution + 10 ml of chloroform, shaking for 3 minutes (condition then should be 1, 2 or 3 above)		

Add the standard zinc solution in 0·1-ml increments, shaking for 1 minute after each addition.

PROCEDURE WHEN THE TEST SOLUTION IS PREPARED AFTER WET-OXIDATION OF THE SAMPLE—

It is sometimes necessary to use wet-oxidation, when ashing the sample is difficult.

Oxidise 1·25 g of the sample in the usual manner, using 1 ml of concentrated sulphuric acid and a measured volume of concentrated nitric acid. Dilute with 2 ml of distilled water, add 8 ml of 5 N ammonium acetate solution, 4 drops of bromocresol green indicator and then cautiously dilute ammonia solution (1 + 9) until the colour just changes to blue. Transfer to a 25-ml calibrated flask and dilute to the mark. Prepare a blank from the same reagents in the same quantities, and finally bring the colour to the same shade of blue.

Use 1 ml of this solution for the approximate estimation and afterwards proceed as described.

NOTES ON METHOD—

1. The volume of the liquids (aqueous and chloroform layers) in the funnels must be kept the same for blank and test and the times of shaking must be observed strictly. The mechanical shaker is almost indispensable.

2. Removal of copper is not necessary unless more than 100 μg are present in the aliquot under test. Lead and bismuth are also "held up." If the copper content in the aliquot of the test solution to be used for the accurate determination exceeds 100 μg, then it may be necessary to remove the copper as follows. Shake volume V of the test solution with excess of dithizone solution. Extract the zinc from the dithizone extract with two 10-ml portions of diluted 5 N hydrochloric acid (1 + 15). Combine the two, add 4 ml of 5 N ammonium acetate solution and 1 ml of 10 per cent. w/v sodium thiosulphate solution and continue as described.

3. It is not possible to match the dithizone colours by ordinary electric light. Daylight is undoubtedly best, but "daylight" fluorescent lighting is good. Colour-matching fluorescent lighting is very good.

4. The colour change is sensitive. It is easy to distinguish 7 μg (say) from 8 μg of zinc; 1 μg of zinc is detectable.

5. As it is not unknown for chloroform to contain traces of zinc, purify it by distilling it over lime in Pyrex-glass apparatus. To stabilise the chloroform add 1 ml of absolute ethanol to 100 ml.

6. The colour changes occurring with 0·5 ml of 0·025 per cent. dithzone solution plus 10 ml of chloroform are as follows—

Amount of zinc, μg	Colour (by transmitted light)
0	Green, tailing (towards the apex of the funnel) to very slight blue
1	Bluish green
2	Reddish violet tailing to blue
3	
4	Reddish violet tailing to bluish violet
5	Strong reddish violet tailing to violet
6	Strong violet-red tailing to reddish violet
8	
9	Strong violet-red tailing to violet-red (purple by reflected light)

Results

Results on various samples by the proposed method and by the Society's method[2] are shown in Table I.

Table I

Determination of zinc in foodstuffs

Sample	Zinc found by proposed method after sample had been—		Zinc found by the Society's method, p.p.m.
	ashed, p.p.m.	wet-oxidised, p.p.m.	
Gelatin "A"	38	43	42
Gelatin "B"	—	—	5
Gelatin "B", with 65 p.p.m. of Zn added	70	79	86
Cocoa "A" (containing about 30 p.p.m. of Cu and 100 p.p.m. of Fe)	61	—	68
Cocoa "A", with 20 p.p.m. of zinc, 10 p.p.m. of lead, 38 p.p.m. of Cd and 40 p.p.m. of Sn added ..	79	—	85

We thank the Directors of Rowntree & Co. Ltd., for permission to publish this Note.

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1. Francis, A. C., *Analyst*, 1957, **82**, 293.
2. Analytical Methods Committee, "The Determination of Zinc in Foodstuffs," *Ibid.*, 1948, **73**, 304.

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First submitted, *March 15th*, 1956
Amended, *October 29th*, 1956

Apparatus

A MECHANICAL SHAKER

TRANSFER of a solute from one solvent to another by shaking under standard conditions in a separating funnel is an operation that can be tedious if the shaking is prolonged and conducted by hand. The mechanical shaker shown in Fig. 1 was designed for the extraction of zinc by a solution of dithizone in chloroform,[1] but it can find general application in those operations in which shaking in a separating funnel is required.

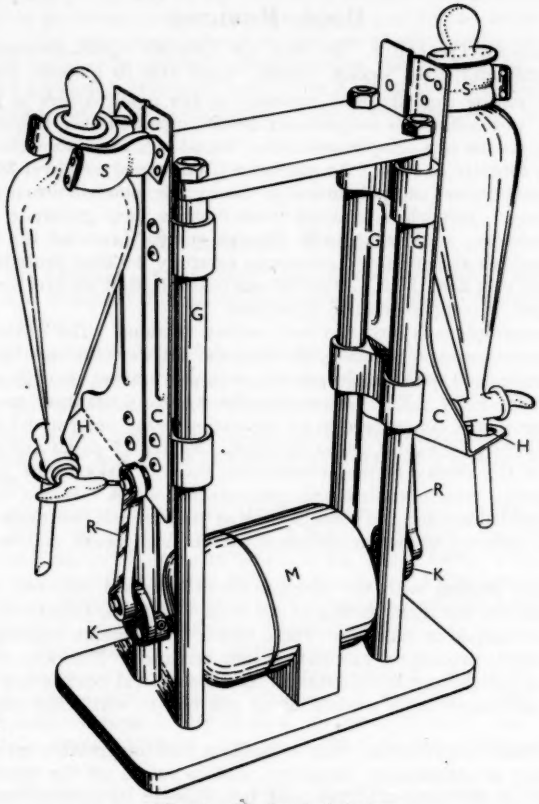

Fig. 1. Mechanical shaker

The conical separating funnels, having a capacity of 150 ml, are borne on the aluminium alloy carriages, C, a cork on the stem of the funnel fitting into the hole, H, in the base of the carriage and the neck of the funnel being held by the steel spring-clip, S. The electric motor, M, preferably geared (230 volts a.c., about 1/20 h.p., 400 r.p.m.) by the crank, K, and connecting rod, R, communicates a reciprocating motion to the carriages, which slide on the vertical steel guides, G. For easy running and good balance, one carriage is at top dead-centre when the other is at bottom dead-centre.

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First submitted, *March 15th*, 1956
Amended, *October 29th*, 1956

British Standards Institution

NEW SPECIFICATION*

B.S. 2824:1957. New Wood Wool for Fillings. Price 2s. 6d.

AMENDMENT SLIP*

A PRINTED slip bearing an amendment to a British Standard has been issued by the Institution, as follows—

PD 2710—Amendment No. 5 (February, 1957) to B.S. 1425:1954. Cleanliness of Fillings and Stuffings for Bedding, Upholstery, Toys and other Domestic Articles.

* Obtainable from the British Standards Institution, Sales Department, 2 Park Street, London, W.1.

Book Reviews

DIE KOMPLEXOMETRISCHE TITRATION. By Prof. Dr. GEROLD SCHWARZENBACH. Pp. xii + 100. Stuttgart: Ferdinand Enke Verlag. 1955. Price DM 19 (paper); DM 21 (cloth boards).

It is difficult to realise that the first reference to the potentialities of polyaminocarboxylic acids ("complexones") as volumetric reagents for metals appeared only 11 years ago, and that the first paper describing the use of a complexone and a "metal-indicator" for determining the hardness of water appeared as recently as 1948. An extensive literature of well over 300 papers now covers the applications of complexones and polyamines to the complexometric titration of an astonishingly wide variety of cations. But while analysts must express their gratitude to chemists such as Flaschka, Přibil, Kinnunen, and Milner, who have so greatly extended the range of application of complexones in analysis and produced numerous carefully detailed procedures, it is still to the originator of the whole vast field, Prof. Gerold Schwarzenbach, that we look for the clear exposition of the basic principles of complexometric titrations.

The present monograph falls into two well defined sections. The author first develops the basic theory of a complexometric titration, showing the manner in which the concentration of a cation diminishes on titration with simple amines, with polyamines, or with polyaminocarboxylic acids of increasing complexity. The various possible types of titration are then considered in some detail. Changes in the concentration of the cation being determined can conveniently be followed during a titration by visual colour changes. Nearly 17 pages are therefore devoted to a sound treatment of the theory of metal-indicators such as Eriochrome black T (Solochrome black WDFA), murexide, metalphthalein and pyrocatechol violet. Redox indicators (variamine blue and dimethylnaphthidine) and the potentialities of photometric titrations are next considered, and several pages are devoted to the simultaneous titration of metals and to the use of masking agents.

After a few pages dealing with the preparation of standard solutions, indicator solutions, buffers and other reagents, the main section of the book (34 pages) consists of a series of detailed procedures for various metals or radicles. These follow the common pattern of detailing applications, reagents needed, procedure, calculation and remarks. This last section is invariably illuminating and gives just the sort of information that the analyst needs when he runs into trouble when trying a new procedure or in modifying an old one to suit some slightly altered set of conditions.

Five pages of "concluding remarks" form a brilliant and imaginative survey of what remains to be done in the field of complexone chemistry, and to round off the whole work there is an excellent bibliography of 181 entries. This book is unique in its content and must find a place in the laboratory of every up-to-date analyst. It is understood that a revised and extended English translation will shortly become available. H. IRVING

SYSTEMATIC HANDBOOK OF VOLUMETRIC ANALYSIS. By FRANCIS SUTTON, F.I.C., F.C.S., and JULIUS GRANT, M.Sc., Ph.D., F.R.I.C. Thirteenth Edition. Pp. xiv + 752. London: Butterworths Scientific Publications. 1955. Price 63s.

"Sutton" has been revised many times in its long career and always the task has been to bring the content matter up to date as regards new technique, but yet at the same time preserve the original concept of the book. Dr. Grant has on this occasion brought about the most thorough change of any edition to date and he must have been severely taxed in deciding how much of the old to discard and what of the modern to include, without completely writing a new book under his own name.

Since the last edition in 1935, there have been enormous strides in general analytical chemistry the volumetric section of which has not lagged behind. The book, indeed, contains much recen

material that is of value and includes very many individual methods that were not present in the previous edition. Information about British Standards for volumetric glassware is always useful in a book of this type, as is the discussion on likely errors in the use of volumetric apparatus. Theoretical considerations underlying volumetric analysis have in this edition been collected together and this assists in reading the book. It must be admitted, however, that the theoretical section of the book is rather superficial, rarely rising above H.S.C. level—or, rather, G.C.E. Advanced Level—with only sidelong glances at modern theory.

Perhaps the only real criticism of the book lies in its omissions, and this impression may of course be a personal opinion. Modern electrometric titration techniques are given scant treatment and they do form a valuable adjunct to the practising analyst's armamentarium. Equally, the newer reagents (such as mercurous nitrate, chlorites and hypochlorites) could have found a larger place in the volume. The wide scope offered by the use of ethylenediaminetetra-acetic acid in volumetric analysis is completely ignored. Even so, this volume lives up to the reputation of its predecessors as an invaluable reference book to be kept in the laboratory. The tremendous amount of work carried out in its compilation is recognised and must be appreciated.

R. F. MILTON

Publications Received

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Errata

MAY (1955) ISSUE, p. 394, 3rd line under "RESULTS." *For* "8·22" *read* "8·42."

MARCH (1957) ISSUE, p. 206. At end of Note by Isaacs, Morries and Stuckey *add* the following address: THE BRITISH DRUG HOUSES LTD., GRAHAM STREET, LONDON, N.1.